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COMPARATIVE NUTRITIVE VALUE OF SOYBEAN MEAL,
SUNFLOWER MEAL, AND MEAT AND BONE MEAL
FOR EGG PRODUCTION

BY

SMART M. ORUSEIBIO

A thesis submitted
in partial fulfillment of the requirements
degree Doctor of Philosophy
Major in Animal Science
South Dakota State University
1985

COMPARATIVE NUTRITIVE VALUE OF SOYBEAN MEAL, SUNFLOWER MEAL,
AND MEAT AND BONE MEAL FOR EGG PRODUCTION

This thesis is approved as a creditable and independent investigation by a candidate for the degree, Doctor of Philosophy, and is acceptable for meeting the thesis requirement for this degree.

Acceptance of this Thesis does not imply that the conclusion reached by the candidate is necessarily the conclusion of the major department.

C. W. Carlson
Thesis Adviser

Date

John R. Romans, Head
Department of Animal & Range Sciences

Date

DEDICATION

I dedicate this thesis work to the memory of my beloved father, Jimbo Oruseibio Okosee, to whom I owe all that I am at present, and will be in the future.

COMPARATIVE NUTRITIVE VALUE OF SOYBEAN MEAL, SUNFLOWER MEAL, AND MEAT
AND BONE MEAL FOR EGG PRODUCTION

ABSTRACT

Smart M. Oruseibio

UNDER THE SUPERVISION OF PROFESSOR C. W. CARLSON

Two experiments were conducted over a period of two years to evaluate the comparative nutritive value of soybean meal, sunflower meal, and meat and bone meal.

Experiment 1 consisted of 864 Babcock--300 pullets randomly distributed into twelve diet treatments. Each treatment was replicated six-times with twelve birds per unit.

Layers diets were formulated at 16, 14, and 12% protein levels with and without amino acid supplementation. The 12% amino acid supplemented level served as the control in the experiment. Soybean meal, sunflower meal, and meat and bone meal were utilized as major sources of protein supplement in yellow-corn based diets.

The parameters measured were hen-day egg production, daily feed consumption, feed efficiency, egg weight, body weight, mortality, Haugh unit, and egg shell thickness.

The experiment lasted for ten 28-day periods, starting January 14, 1981, and terminating October 22, 1981.

The results of the experiment as measured by the collected data on the criteria stated above showed comparable egg laying performance among hens fed soybean meal and sunflower meal diets. Performance of hens fed meat and bone meal diets was inferior compared to hens fed

either soybean meal or sunflower meal diets in hen-day egg production, feed intake, and feed efficiency, and egg weight ($P < 0.05$).

Experiment 2 was conducted to explore the possibility of utilizing meat and bone meal beyond its traditional role of supplementing calcium and phosphorus in egg laying diets in small amounts. The study involved 720 Shaver-288 pullets randomly distributed into ten dietary treatments, each treatment was replicated six-times in twelve bird units.

Diets were formulated at 16% and 12% protein levels with amino acids supplemented at the 12% levels.

Soybean meal, sunflower meal, and meat and bone meal by themselves alone, or mixtures of either meat and bone meal with soybean meal or sunflower meal were utilized as major sources of protein in yellow-corn based diets. The same parameters as in Experiment 1 were measured. The experiment lasted for thirteen 28-day periods, starting February 26, 1982, and terminating February 24, 1983.

The results indicated poor performance of hens fed meat and bone meal diets, and comparative performance of hens fed soybean meal and sunflower meal diets. The performance of hens fed mixed diets of sunflower meal with meat and bone meal was at par with those fed either sunflower or soybean meal diets. The performance of hens fed mixed diets of sunflower meal and meat and bone meal was significantly better than those of soybean meal with meat and bone meal. ($P < 0.01$)

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I wish to thank each and everyone of my committee members, sincerely, for their constructive critique of this manuscript.

I am most grateful to my wife Elizabeth, and my children whose support, understanding, and prayers have carried me throughout my Ph.D. career in South Dakota. They gave me hope and inspiration to overcome all the insurmountable barriers.

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SMD.

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INTRODUCTION

A challenge to the animal nutrition scientist from the beginning of animal agricultural husbandry practices for providing animal products like milk, meat, and eggs has been the search for least cost animal ration formulations. Thus, this challenge is a continuous one because changing world economic situations frequently affect cost of ingredients. Rations that supported optimum production performance for reasonable economic returns in the past may be found less suitable today because of price changes.

The search for least cost formulation has led the animal nutritionist to investigate the nutrition potential in common ingredients used for compounding animal and poultry rations, and at what percentage of combinations such ingredients could furnish satisfactory support for maximum production performance. Examples of such research is seen in the studies reported by Bray (1960); Bray and Garlich (1960); and Bray (1964a,b; 1965; and 1968a). Similar reports are found in the literature by several other investigators of such studies.

The protein component of animal rations is the most expensive portion; it represents about 65-75% of the total cost of the ration. The nutritionist has more frequently spent his energy and time in searching for alternate cheap sources of protein that could furnish adequate nutrition for optimum production performance. This led to the earlier intensive studies of minimum protein levels that could furnish optimum production performance.

Such studies were centered on the following areas:

1. Minimum protein level for satisfactory egg production performance (Hill and Anderson, 1955; Thornton et al., 1957; Talley and Sanford, 1966; Ross and Herrick, 1976).
2. Amino acid requirements and supplementation of low protein levels for optimum egg production performance (Almquist et al., 1935; Gray and Peterson, 1946; March et al., 1950; Johnson and Fisher, 1956, 1958, 1959; Heywang, 1956; Bradly and Quisenberry, 1961; Stangeland and Carlson, 1961; Britzman and Carlson, 1964).
3. Adequate energy-protein ratios for optimum egg production performance (Miller et al., 1957; Berg and Bearse, 1957; Hochreich et al., 1958; Frank and Wassel, 1960; Hill et al., 1960; and Thornton and Whittet, 1960).
4. Influence of breed, age, and strain differences on protein requirements (Milton and Ingrams, 1957; Harms and Waldroup, 1962; Aitken et al., 1973; and Hamilton, 1978).
5. Effect of environmental temperature on protein requirement (Bray and Gessel, 1961; and Zablan et al., 1963).
6. Absorption of amino acids (Wheeler, 1958; Tasaki, 1966; Wiseman, 1968; and Kan, 1975).
7. Amino acid interactions (Gray, 1947; Jones, 1964; Boorman and Fisher, 1966; and Featherston, 1975, 1976).
8. Effect of heat on protein quality (Mussehl, 1942; Morrison et al., 1953; and Bornstein et al., 1961).

Because of the expensive cost of animal protein supplements in compounding animal rations, the nutritionist has to resort to utilization of vegetable sources of protein whenever the use of animal protein supplement is not a critical necessity. Plant or vegetable sources of protein are relatively cheap compared to animal protein supplements. Among the common vegetable or plant sources of protein utilized in animal rations are soybeans and soybean meals, cottonseed meal, peanut meal, rapeseed meal, and sunflower seeds and sunflower meal. The energy part of the ration is largely furnished by the cereal grains; but occasionally, either animal fat or vegetable oil is added to furnish the desired energy level.

According to the Food and Agricultural Organization (F.A.O.) report of 1976, soybeans are the most popular vegetable crop grown world-wide followed by sunflowers. Soybean protein generally has a higher biological value compared to the other vegetable proteins, and it is used in animal rations.

Reports on feeding of extracted raw soybean meal or unextracted raw soybean meal for satisfactory egg production performance were contradictory. For example, Carver et al. (1946); Fisher et al. (1957); Griminger and Fisher, (1960); and Saxena et al. (1963a,b) reported respectively that raw soybean meal supported satisfactory egg production performance. But on the contrary, Hill and Renner (1963) and Rogler et al. (1964) respectively reported negative results.

Reports of growth trials with chicks, turkey poults, swine and rats (all monogastric animals) indicated that raw soybean has a growth

depression effect: for poultry (Ham and Sandstedt, 1944; Ham et al. 1945; and Lepkovsky et al. 1965); for swine (Osborne and Mendel, 1917; and Jimenez et al. 1963); and for rats (Morgan and King, 1936; Borchers et al. 1947; Borchers et al. 1948; and Borchers, 1958, 1961, and 1965). Reports also indicated that in both chicks and rats amino acid supplementation plus vitamins counteracted the growth inhibition effect (Fisher and Johnson, 1958; Fisher and Shapiro, 1963; for poultry; and Borchers, 1961; for rats). However, Saxena et al. (1963); and Hill et al. (1953) did not find any beneficial effect of amino acid supplementation in counteracting the growth depression effect of raw soybean meal. Further reports also indicated that with age, chickens can out-grow the susceptibility to the growth depression effect. Fisher and Shapiro (1963) indicated that swine lacked that mechanism. Bornstein et al. (1961); Fisher et al. (1957); Alumont and Nitsan (1961); Saxena et al. (1963a,b); and Woods et al. (1971) respectively, indicated that chickens out-grow the growth depression effect of raw soybean meal. But on the contrary, Bornstein and Lipstein (1963) held the view that age had no influence on chick's susceptibility to the raw soybean meal growth inhibition effect.

Several reasons have been reported for the inferiority of raw soybean meal in monogastric animal nutrition compared to heat treated soybean meal such as unavailability of methionine compared to other amino acids; (Almquist et al. 1942; Evans and McGinnis, 1946; Fisher and Johnson, 1958; Baldini, 1961; Smith and Scott, 1965), and interference with normal growth in animals (Liener, 1962).

But Mitchell et al. (1945) reported that digestibility of raw soybean meal is only slightly lower than that of heat treated soybean meal, while the biological value is much lower. Nitsan (1965) indicated equal apparent digestibility of methionine between raw soybean meal and heat treated soybean meal. Nitsan (1965) reported equal biological value between heat treated soybean meal and raw soybean meal (49.59% vs. 49.21%), but indicated a higher true digestibility for heat treated soybean meal than raw soybean meal (88.26% vs. 64.27%). Hayward et al. (1936); Evans, (1944); and Carroll et al. (1952); indicated that growth differences among animals fed raw and heat treated soybean meals could not be a true measure to account for differences in digestibility of raw and heat treated soybean meals.

While raw soybean meal is a problem in young subjects, it is not a problem in older subjects. As already stated above, raw soybean protein meal in egg laying rations did not show any adverse effects. Recently, Crenshaw and Danielson (1985), have reported excellent performance of raw soybean meal in gestating and lactating swine and indicated that gilts fed raw soybean meal diets showed an increase in litter birth weight ($P < 0.05$) as compared to that from regular commercial soybean meal, and also had higher milk fat at day three of lactation.

Several reports have indicated the favorable effect of moderate heat in treating soybean meal on its nutritional value (Fritz et al. 1947; Clandinin et al. 1947; Alexander and Hill, 1952; and Morrison et al. 1953). Reports also have indicated that higher temperature treatments were much more beneficial in ruminant nutrition than

monogastric animal nutrition (Sherrod and Tillman, 1962; Glimp et al. 1967; and Plegge et al. 1982).

Soybeans have also been fed as soy-flour in milk replacement diets to early weaned piglets (Lennon et al. 1971) and for calves (Colvin and Ramsey, 1968, 1969). The soy-flour meal was either acid or alkali treated, and the report indicated excellent performance comparable to whole milk.

A number of reports have indicated a growth stimulatory effect in soybean meal; i.e., an unidentified growth factor (Hill, 1948; Savage, 1950; Kratzer et al. 1958, 1959; and Wilcox et al. 1961a, b). Such reports were based on data from growth trials with chickens and turkey poults. On the other hand a number of deleterious effects also have been reported in raw soybean meal (McCarrison, 1934; and Patton et al. 1939--goiter; Liener and Pallansch, 1952--toxic substance [Soyin] and haemagglutin factor; Saxena et al. 1962--increased oxygen consumption; and Carlson et al. 1963a, b--rachitogenic effect.)

The consensus of opinion from all available scientific data in evaluation of soybean protein is that it is deficient in the sulfur amino acids; methionine is the first limiting amino acid. The deficiency of methionine in soybean protein notwithstanding; soybean has stood the test of time as number one vegetable protein supplement in animal diets.

Sunflowers are the second largest crop grown world-wide (F.A.O. reports 1976). The Russians and Canadians have long been using sunflower meal in animal rations. According to Morrison, 1949;

sunflower seed cake and forage were commonly used in European countries because of its cheapness; and earlier Tabakoff (1939) indicated that sunflower meal was economically utilized for poultry rations under Bulgarian conditions.

Americans generally have not considered the use of sunflower meal protein supplement in animal rations. This is due partly to earlier reports of variability of the nutritional value of sunflower meal, because of high temperatures used in processing the meal then, (Morrison, et al. 1953). Cullison, (1979), indicated that sunflower meal is not as good as soybean meal for supplementing farm grains for non-ruminant animals.

But in the 1970's as indicated by Jensen et al. (1970), the sunflower acreage trippled in the United States of America (U.S.A.); and production also switched from medium oil yielding crops for traditional use by the confectionary bakery industry and as seed for birds to high yielding varieties for oil extraction. Thus increasing production, particularly in the midwest areas of Minnesota, North and South Dakota aroused interest in utilization of sunflower meal in animal rations as a by-product of sunflower seed oil extraction. Accordingly, the American Feed Manufacturers' Association in 1980, in one of their resolutions, suggested animal nutrition scientists investigate the nutritional value of sunflower meal in poultry and animal rations.

Earlier studies with chick and poult growth trials and laying hens were somewhat contradictory. Mitchell et al. (1945) reported a poor biological value for sunflower meal protein compared to heat

treated soybean meal protein. Grau and Almquist, (1945) reported that sunflower meal furnished all the essential amino acids to support chick growth; but McGinnis et al. (1948) reported to the contrary that sunflower meal is deficient in lysine, but rich in methionine. The data of McGinnis et al. (1948) was supported by several investigators (Harvey, 1956; Klain et al. 1956; Thomas et al. 1965; and Vaughan, 1970). The only two reports on turkey poult growth in evaluating sunflower protein's nutritive value also were contradictory. Slinger et al. (1949) reported a satisfactory growth performance while Garthy et al. (1950) reported otherwise.

Reports on egg production performance also indicated contradictory views. Hale and Brown (1957) and Walter et al. (1959) reported satisfactory performance by egg laying hens on sunflower meal as the major source of protein supplement. But Rose et al. (1972) reported depressed egg production in hens fed sunflower meal compared to soybean meal.

Shyam and Sell (1963) reported equal nutritive values of sunflower meal and soybean meal greater than that of rapeseed meal and animal tallow in a comparative study with growing turkeys. The protein supplements were added in the diets at 5% and 10% inclusion rates. Rapeseed at 5% and 10% rates depressed feed consumption and feed efficiency, and consequently, resulted in less weight gain. Similar results with rapeseed were reported by Sell and Hodgson (1962) in chick growth trials.

Uwayjan et al. (1983) recommended that not more than 30% sunflower seeds be used in laying rations; Rad and Keshavarz (1976) indicated that not more than 50% of the soybean meal be replaced by sunflower meal in broiler rations.

Sunflower protein has been fed as seeds and as a meal to growing-finishing swine and as seeds to gestating swine. Delic et al. (1964) indicated lysine deficiency was evident in sunflower diets fed to growing-finishing swine; and Cunha (1957) indicated that not more than 20-30% of the total growing-finishing swine ration should be sunflower meal, because higher rates depressed feed intake.

Laudert and Allee (1975), Dinusson et al. (1980 a,b), and Hartman et al. (1985) respectively, fed sunflower seeds to growing-finishing swine and reported decreased feed intake with improved feed efficiency and rate of gain. The effects on carcass quality were similar to that obtained with a cooked soybean diet, a decrease in carcass firmness with a higher content of linoleic acid compared to other unsaturated fatty acids. Keppler, (1981) reported no dietary effect of feeding sunflower seeds to gestating sows on feed intake.

The consensus of scientific opinion in the evaluation of the nutritive value of sunflower protein in swine nutrition is that with the exception of lower lysine, there is little difference between sunflower meal and soybean meal. Several feeding trials have demonstrated the beneficial effect of supplementing sunflower meal with synthetic lysine to equalize the amount of lysine in soybean meal

(Russon et al. 1972; Nielsen and Aherne, 1981; Thacker et al. 1984; and Jorgensen et al. 1984).

Hartman et al. (1985) reported through a taste panel that sunflower meal fed pork was acceptable to consumers.

Favorable reports on the nutritive value of sunflower meal have been reported in studies with fattening steers, (Pearson et al. 1954) and in fattening lambs, (Amos et al. 1974). Pearson et al. (1954) indicated comparable nutritive values for cottonseed meal and sunflower meal; and Amos et al. (1974) reported superior performance of lambs fed sunflower meal replacing soybean meal.

While there are no known deleterious effects in sunflower meal according to Jurgens (1978), a number of organic acids have been reported in sunflower meal which could cause staining of eggs. Millic et al. (1968), Mikolajczak et al. (1970), and Rose et al. (1972), reported the incidence of chlorogenic acid. However, these acids have a beneficial effect in ruminant nutrition, because they behave in a manner similar to tannic acid, and thus prevent the microbial proteolytic action on sunflower protein in the rumen.

According to Kratzer and Davis (1959), meat and bone meals vary in feeding quality because of the very nature of being by-products of the meat packing and rendering industries, and also the variability of heat treatment used during the processing procedure. A number of reports have indicated deficiencies of the sulphur amino acids and tryptophan in meat and bone meal due to high gelatin and connective

tissue contents (Almquist et al. 1935; Kraybill and Wilder, 1947; Bock and Weiss, 1956; and Kratzer and Davis 1959).

An evaluation of the protein quality of meat and bone meal was based on chemical analysis indices. Fraenkel-Conrat and Cooper (1944) reported that orange-G-dye (1-phenyl-azo-2 naphthol-6,8 disulfonic acid sodium salt) combines stoichiometrically with free amino imadazole and guanidyl groups in an intact protein. Thus, overheating renders the free amino groups unavailable to react with the dye. Therefore, the dye-binding capacity of meat and bone meal is a good measure of its feeding value as influenced by overheating.

Carpenter (1960) used fluordinitrobenzene to measure lysine availability in meat and bone meal; and Choppe and Kratzer (1963) used both hot water solubility and orange-G-dye-binding capacity to measure the feed value of meat and bone meal and indicated a positive correlation ($r = +0.68$) with chick growth ($P < 0.01$).

Buyan (1959) reported meat and bone meals to bind less orange-G dye than expected from their protein content as an indication of overheating. Buyan and Price (1960) found a relationship between orange-G dye binding capacity of meat and bone meal and methionine utilization as indicated in a rat assay.

Johri et al. (1980) felt rightly that while the chemical analyses were quicker, they often did not correlate with chick growth. Therefore, they used a chick growth assay as a much better indicator for measuring the protein quality of meat and bone meal.

Cullison (1979) suggested that meat and bone meal should not be used in poultry and swine diets at levels more than 5% because of its high calcium and phosphorus content and deficiency in some essential amino acids. McDonald et al. (1973) suggested that meat and bone meal be limited in laying hens and fattening pig diets. They cautioned that meat and bone meal is less acceptable to ruminants and also indicated that meat and bone meal is limited in methionine and tryptophan.

In conclusion, digestibility of a protein is not necessarily associated with its nutritional value, as all the digestible material may not be biologically available; and differences in the rate of liberation and subsequent absorption of amino acids in the gut may affect the efficiency of utilization of different proteins.

OBJECTIVES

The objectives of the study were as follows:

1. To re-evaluate the nutritional value of sunflower protein versus soybean meal in the light of presently available high oil yielding sunflower seeds.
2. To evaluate at what minimum protein level sunflower meal could support satisfactory egg production performance.
3. To determine if meat and bone meal could be used in larger proportions in practical layer diets beyond the traditional lower levels.
4. To evaluate the combinations of any of the three protein supplements in yellow-corn based layer diets.

LITERATURE REVIEW

I. CRUDE PROTEIN LEVEL REQUIREMENTS FOR EGG LAYING HENS.

Feed costs about 75% of the total cost of egg production and protein supplements are the most expensive portion of the feed component. Therefore, the subject of adequate or proper nutrition for the egg laying hen, being provided at a minimum cost, has always occupied the attention of the poultry nutrition scientist.

Earlier reports on levels of protein adequate to support maximum egg production were controversial and contradictory. Heuser (1941) and Heuser et al. (1945) respectively, reported that a 15% crude protein level in laying hen diets was sufficient enough to support maximum egg production, egg size, and body weight maintenance. Bray and Gassel (1961) showed the adequate daily protein intake to be 12 gm. and that any intake below that results in decreased egg production. Aitken et al. (1973) reported an average daily protein intake of 17 gm. in the laying year. Balloun (1969) in his study of the effect of housing density on the protein requirement of laying birds indicated that with one bird per cage egg production was satisfactory on a 12% protein level, but that with two or three birds per cage upwards of 16% protein was required for maximum egg production.

However, Milton and Ingram (1957) reported that an 18% crude protein level in laying rations was superior to either a 16 or 14% protein level. Further, they reported that a 14% protein meal was adequate only for pullet egg layers and not for old hen layers. Hochreich et al. (1958) reported 17% protein to be the required level

for maximum egg production. Frank and Waibel (1960) reported 14.9% protein to be satisfactory. Thornton et al. (1957) presented data showing 13% to be adequate when layers are kept in cages. Thornton and Whittet (1959, 1960) also indicated that a 13% protein diet was comparable to ones with higher protein levels for egg production and feed efficiency. Miller et al. (1957) showed satisfactory egg production performance with a 12.5% and 13% protein respectively. Talley and Sanford (1966) used a 14%, 15%, 17%, and 18% protein respectively and indicated that the higher levels enhanced egg production performance under high temperatures and senescence. Deaton and Lillie (1959) reported maximum egg production performance from protein levels above 15% as did Quisenberry and Williams (1952) and Quisenberry and Bradley (1962). Malik and Quisenberry (1963) in their study of the effects of feeding milo or corn and protein levels on egg production performance reported that an 18% protein level was superior to a 15% protein level. Bradley and Quisenberry (1961) reported superiority of an 18% protein level to a 16 and 14% for egg size with 16% showing intermediate and 14% the smallest egg size. When they supplemented those protein levels with methionine and lysine, they found only lysine to give a response. Depressed egg production was noted with the 18 and 16% levels (not significant) while maximum egg production occurred with the 14% protein level.

Some nutritionists reasoned that higher protein levels would be required in high energy diets and increased rates of egg production. They reasoned this on the premises of Wilcox (1934) who found that the

protein required for egg formation came entirely from the diet. Therefore, a hen on a high energy diet would consume less feed, and thus, would need a diet with higher protein. Accordingly, Reid et al. (1951) reported the superiority of an 18% protein diet to either a 15% or 13% high energy diet. Similarly, Ingram (1956) indicated the superiority of a 17% or 19% protein diets to a 15% diet for caged layers.

Contrary to the above findings, Heywang et al. (1955) showed that performance with a 15% protein level in a high energy diet was comparable to that for egg production under both hot and moderate weather conditions. Hill and Anderson (1955) also found that a 15% protein ration was adequate for maximum egg production in high energy diets fed to White Leghorns.

Further, they also found that the protein requirements for a smaller strain of White Leghorns was between 15 and 16.5% under similar experimental conditions. They added 5% fat to the ration and found that the additional energy incorporated did not elicit an additional protein need but did improve egg production. Thus, their findings indicated that an increased level of dietary energy from fat enhanced utilization of dietary protein. Miller et al. (1956) also found no difference in egg production and body weight maintenance by increasing the dietary protein level from 12% to 21% and the energy level from 640 to 930 productive energy calories per pound of diet. However, they reported an association of increased feed efficiency with increased energy. Further, Berg and Bearse (1957) showed that hens on high energy diets with 14% protein performed less efficiently than hens on

low energy diets with the same protein level. McDonald (1938) reported that 12.5% protein layer diets compared favorably with 15% protein level diets as to egg production potential. Carpenter *et al.* (1954) compared the effect of dietary protein level on egg production performance of hens on all-vegetable diets or vegetable and animal protein diets. They used fish meal as the protein supplement to the basal diet and found that an 11% crude protein level supported egg production equal to that of higher levels. Finally, Ross *et al.* (1976) showed no significant difference in egg production performance of layers on 14% protein levels versus 16% protein levels, with no effect of dietary protein level on egg shell thickness or specific gravity. Also, Hamilton (1978) reported no dietary protein level effect on kg. feed/dozen eggs, but that protein had a significant effect on kg. feed/kg. eggs. They further showed that there was no protein level by strain interaction and no dietary protein level effect on mortality.

II. THE EFFECT OF HEAT PROCESSING TREATMENT ON PROTEIN QUALITY

Processing animal and poultry feed stuffs with heat treatment has both beneficial and adverse effects, particularly when excess heating occurs. Heat temperatures beyond certain limits in processing soybean meal, sunflower meal, and meat and bone meal have resulted in reduction of the nutritive value of the protein. Thus, several workers have carried out studies on the effect of heat on protein quality of these feeds to assess the optimum temperature that would affect maximum protein quality as measured through biological assays.

Clandinin *et al.* (1947) reported on a study with White Leghorn chicks fed soybean meal processed at 6.8 kg. pressure for four to eight hours duration and indicated deficiencies of known vitamins, lysine, arginine, tryptophan, and methionine. Their results agreed with those of Bird and Burkhardt, (1943) who evaluated the nutritive value of soybean flakes by autoclaving at 9.09 kg. pressure for varying lengths of time.

Wilgus *et al.* (1936) in their biological assay study of growth in chicks with heated soybean meals indicated that the maximum heat treatment with the expeller process should be between 284°F and in the hydraulic process 221°F for ninety minutes in the cooker and in the solvent extraction process 208.4°F for fifteen minutes. Their results agreed with those of Hayward *et al.* (1937) who reported 221°F-249.8°F for two and a half minutes in the expeller process, 221°F-249.8°F for ninety minutes in the hydraulic process and 208.4°F for fifteen minutes in the solvent extraction process. Growth of chicks was doubled that

fed on poorly heated or raw soybeans. Further, they claimed that the improvement in the nutritional value correlated with the availability of the sulphur amino acids in raw soybean protein.

Fritz et al. (1947) with turkey poults showed evidence that the best nutritive value of ground raw soybeans is obtained with 6.8 kg. pressure for 20-30 minutes of autoclaving. Further, they indicated that dry heat was not as desirable as moist heat. Dry heat caused damage to the protein of the soybean which was not corrected by lysine and methionine supplementation. Similar damage could be caused by excessive moist heat as well. Conclusively, they said that autoclaving at 6.8 kg. pressure could only become injurious when continued beyond one and a half hours. Hayward and Hafner (1941) substantiated that claim.

Mattingly and Bird (1945) carried out growth trial studies with New Hampshire chicks on the recommendations of Bird and Burkhardt (1943) and found that drying the soybean meal to two percent moisture content did not interfere with the effect of autoclaving on the nutritive value of the meal. But, they also reported that drying the soybean meal to a constant weight at 221°F did interfere with the subsequent beneficial effect of autoclaving on nutritive value.

Parsons and Walliker (1941) also indicated that moist heat had a greater beneficial effect on the quality of soybean protein than dry heat. However, Parsons (1943) reported that the beneficial effect of moist heat on soybean protein quality was possible only under moderate

heating temperature conditions and that it could produce adverse effects also if autoclaving was prolonged or the pressure raised.

Hayward et al. (1936) and Johnson et al. (1939) working with growth rate assays in rats, and Evans and McGinnis (1946) working with chick growth explained the beneficial effect of heat treatment on soybean protein as being the unlocking of the sulphur amino acids in raw soybeans, which are unavailable to monogastric animals. Evans and McGinnis (1946) indicated that the availability of the sulfur amino acids was improved at temperatures of autoclaving of 212°F, 230°F, or 248°F and that above 248°F for 30 to 60 minutes destroyed the availability of these amino acids. Their findings agreed with those of Hayward et al. (1936); Hayward and Hafner (1941); Almquist et al. (1942); and Bornstein et al. (1961).

Morgan and King (1926) reported adverse biological value in a study with rats fed cereal and casein proteins treated with dry heat at 392°F and 302°F for a duration of thirty to forty-five minutes. Greaves et al. (1938) duplicated Morgan and King's work to verify the temperatures at which adverse biological value of the proteins occurred on such heat treatments. They experimented with rats on casein protein and found that heating the casein at 212°F for eight hours or at 248°F for two hours did not affect growth rate, but heating at 248°F for eight hours or more could reduce growth rate. Heating at 266°F for thirty minutes caused perceptibly reduced growth, and such reduced growth was progressively pronounced as the heating time was lengthened to two, eight, and twenty-four hours. Their findings agreed with the

earlier results of Morgan (1931) and Morgan and Kern (1934) respectively.

Alexander and Hill (1952) studied the effect of heat on lysine and methionine in heat treated sunflower meal. They autoclaved the meal at 6.8 kg. pressure and applied dry heat at 249.8°F respectively and indicated that lysine was largely destroyed; but there was no effect on methionine. Thus, they concluded that destruction of lysine during processing is the probable major cause of the poor nutritional value for chick diets of commercial expeller processed sunflower meal.

Morrison *et al.* (1953) showed that processing conditions affected the nutritive value of sunflower meal. Nutritive values of sunflower meal increased with lowered processing temperatures, and that opening the choke on the expeller produced superior sunflower meals than similar conditions when the choke is in regular position. Finally, they showed that sunflower meal of equivalent nutritive value to solvent extracted soybean meal can be produced by lowering the temperature in the cooker from 464°F to 392°F and from 500°F to 428°F in the conditioner.

Glimp *et al.* (1967) reported a study on growth rate, digestibility, and nitrogen retention with crossbred twin lambs fed either a 12% protein diet with soybean meal dry heat treated at 300.2°F for four hours or a control group with 17% protein diet with soybean meal without heat treatment. Their data indicated superior performance of the lambs on the the 12% protein to that of the 17% non-heat treated group and attributed the beneficial effects due to reduced microbial

ruminal degradation of the heat treated protein. The heat treatment reduced solubility of the protein from 72 to 35%.

Becker et al. (1953) reported the effect of heat treatment on the nutritive value of soybean meal in swine nutrition. They carried out a growth trial study on some sixty-four pigs of weaning age with hexane extracted-desolventized, hexane extracted-partially toasted, hexane extracted-regularly toasted, or hexane trichloroethylene extracted-regularly toasted soybean meals and found that all except the desolventized group were significantly inferior in growth rate. This explained the beneficial effect of heat treatment on the nutritive value of soybean protein. However, Robison (1951) reported that a "moderately" toasted solvent soybean oil meal was slightly inferior to an expeller soybean oil meal.

Plegge et al. (1982) studied the effect of heat treatment of soybean meal at temperatures of 215°F, 262°F, 291.2°F, 318.2°F, and 372.2°F in ruminants and indicated that heat roasted soybean meal at either 262°F or 291.2°F increased better utilization of soybean meal. At 318.2°F or 365°F nitrogen retention increased. Chick biological assay studies showed no difference in growth rate with either 262°F or 291.2°F, but soybean meal roasted at 318.2°F depressed growth rate in the chicks.

Sherrod and Tillman (1962) studied the effect of heat on the nutritive value of cottonseed and soybean protein for digestibility and nitrogen retention using growth trials in sheep and rats. The proteins were subjected to three treatments: (1) no heat, (2) autoclaved

under 6.8 kg. pressure at 182°F for forty-five minutes, and (3) autoclaved under the same pressure for ninety minutes. Their results indicated that the two heat treated groups did not differ as to fecal nitrogen loss (increased), urinary nitrogen loss (decreased), and nitrogen retention (increased). Heat reduced ruminal ammonia levels and plasma non-protein nitrogen levels. The 45 minutes heat treated proteins produced significantly better weight gains and feed efficiency compared to the non-heat treated control group. The rat growth trials indicated that conditions of processing to effect optimum nutritive value of cottonseed protein should be different from those of ruminants.

Several other workers such as Sharpless et al. (1939); Mussehl (1942); Ham et al. (1943); Michell et al. (1945); Klose et al. (1946); Menick et al. (1946); Block et al. (1946); Pader et al. (1948); Altschul (1958); Ascarelli and Gestetner (1962); Featherston and Rogler (1966); Combs et al. (1967); and Arnold et al. (1971) reported the beneficial effects of moderate heat treatment on the nutritive value of soybean protein.

Klose et al. (1946) reported the inactivation of growth inhibiting substance in raw soybeans with heat treatment. Ham et al. (1945) and Menick et al. (1946) respectively, reported the release of essential amino acids by hydrolysis upon proper heat treatment of soybean protein. Wilgus et al. (1936) and Sharpless et al. (1939) reported inactivation of goitrogencity activities in soybean upon heat treatment and also by action of fat solvents.

Hayward (1937) reported that the solvents used in commercial extraction of soybean meal had no effect on the quality of the soybean protein.

III. FACTORS AFFECTING PROTEIN LEVELS IN EGG LAYING RATIONS

A close examination of the results of various early workers who investigated the adequacy of protein levels in laying rations reveals that there were a number of factors some of them did not consider before reporting their findings. The problem of minimum protein level for maximum egg production lies largely on the biological value of the protein in terms of essential amino acids and balance and also on the source of protein supplement, environmental temperature, and the strain of the bird. Thus, much of the controversial and contradictory reports were put to rest, when later investigations based on the above criteria satisfactorily showed that, with proper supplementation of deficient amino acids, a low protein diet supported egg production equal to that of a high protein diet. Under high environmental conditions, higher protein levels are desirable for maximum egg production performance; and that finally, there is a genetic interaction among breeds or strains of birds and the optimum protein level for maximum egg production.

Thus, Johnson and Fisher (1959) showed that a 10.4% protein diet plus adequate supplementation with methionine and lysine supported egg production equal to that of a 15.7% protein diet. Quisenberry (1965) reported that methionine is the first limiting amino acid in egg laying diets composed mainly of corn and soybean meal, and that proper supplementation of low protein corn-soy diets with methionine improved egg production. Griminger and Fisher (1959) showed evidence of the importance of proper balance of amino acids in egg laying rations.

They augmented a low protein diet of 10.4% protein level with gelatin, zein or glutamic acid at a 2.5% inclusion rate and compared it with a control diet of 15% protein. They indicated that hens on the diet with the gelatin addition performed equally to those on the 15% protein in terms of egg production and body weight maintenance. But both the zein and glutamic acid supplemented diets supported poor performance compared to the control. They attributed the cause to an amino acid imbalance and concluded that, amino acid balance is an important factor in the protein nutrition of laying hens on low protein diets. Thornton *et al.* (1957) suggested the need for increased protein levels in high environmental temperature. Fernandez *et al.* (1973) reported no significant difference in egg production performance from rations consisting of 11.5%, 13%, 15%, 16.3%, and 18% protein levels, respectively, when the lower protein corn-soybean diets were adequately supplemented with lysine and the sulfur amino acids. Harms *et al.* (1962), and Harms and Waldroup (1963) indicated that methionine hydroxanalog Ca(MHA) supplementation of diets containing 13% protein improved feed utilization and egg production performance of laying hens. Bray and Garlich (1960) reported that supplementation of a 9% protein corn-soybean diet for laying hens with amino acids, equivalent to the amount suggested by Johnson and Fisher (1959) produced significant increase in egg production and nitrogen retention. However, they failed to get a response in another experiment in which a 9.4% protein level was supplemented and compared to the control 16% protein ration. Adams *et al.* (1958) used corn-soybean meal diets on White Leghorn layers and found

that a 12% protein level gave as satisfactory egg production as higher protein levels when all known vitamins were supplemented. They carried out a second experiment with a 10% and 12% protein levels and a third experiment at the 10% protein level with addition of all known vitamins, and the results were essentially the same. Thus, they concluded that addition of more vitamins to a 10% corn-soybean diets may be beneficial. They made an overriding statement that a protein level between 12 and 14% in corn-soybean meal diets for Leghorn layers in floor pens was optimum for satisfactory egg production.

Naber and Touchburn (1963) utilized three methods of supplementing low protein diets with amino acids. They supplemented a 12% protein diet to provide the National Research Council (NRC) (1960) minimum requirements and obtained a response from lysine but not from methionine. A comparison of the amino acid patterns of the 12% and 16% protein diets showed negative deviations in the amino acid pattern of the 12% protein diet for lysine and arginine. Egg production improved when lysine alone was supplemented, but the combination of lysine and arginine supplementation depressed the rate of lay. When they used the amino acid profile of the whole egg as a basis for supplementing the low protein diets, they found responses from lysine, threonine, tryptophan, and valine when supplemented in the appropriate combinations.

Britzman and Carlson (1963) obtained the following results in supplementing low protein diets:

1. A significant increase in hen-day egg production was obtained with lysine and methionine supplementation in a high energy diet.

2. Methionine only elicited a significant response in a low energy diet.

3. By using a combination of glycine, valine, arginine, isoleucine, tryptophan, lysine, and methionine, they obtained a significant increase in egg production. However, a third experiment showed that this response was due to tryptophan, lysine, and methionine and not the combinations of amino acids.

Deaton and Quisenberry (1964) reported that layers which received a 14% protein diet with yellow-corn as the grain portion of the ration significantly out-performed those which received sorghum grain. Further, they showed that hens which were fed a 16% protein diet of animal and plant protein were significantly superior to hens which received 14% all plant protein. The mixed protein group had much better feed efficiency.

Deaton and Quisenberry (1965) reported a significant improvement in body and egg weights with hens which received a 14% sorghum protein diet when it was supplemented with lysine, methionine, tryptophan, threonine, and arginine. Further, they also found that there was no significant difference between the two groups in respect to hen-day egg production and feed efficiency; although the hens with the amino acid supplemented diet showed a 2.25% non-significant increase in hen-day egg production. Finally, they indicated that there was no

beneficial effect from amino acids supplementing a corn diet and that the corn diets supported significantly higher egg production, egg weight, and feed efficiency with or without amino acid supplements. However, Berry (1958) found no significant difference in milo and corn all-mash diets, although his results also indicated a slightly higher egg production performance in favor of the corn diets. Further, Adolf and Grau (1956) compared commercial layers in five California ranches for two six-week periods. They fed a 17.43% protein predominantly corn ration and an 18.03% protein predominantly milo ration and found no differences between the two treatment groups in egg production.

The third criteria listed as one of the possible sources of the controversy in earlier reports of establishing the adequate protein level for maximum egg production was environmental temperature. Understandably, some of the early workers were less aware of the influence of environmental temperature upon voluntary food intake. Layers consume more feed during cold seasons and less feed during hot seasons. Thus, this knowledge is utilized in formulating diets with optimum protein levels to reflect the seasonal variations.

Bray and Gessel (1961) studied the effect of environmental temperatures upon suboptimal levels of protein on egg production performance. They fed White Leghorn pullets corn-soy based diets, where corn furnished 45% of the total protein, at protein levels of 11.5, 12.0, and 14.0%. They carried out two experiments. The first conducted at 42°F and the second at 76°F and 86°F each for an eight-week assay period. They indicated that the extreme temperatures of 42°F and 86°F

altered feed intake but did not affect the rate of lay provided protein intake was adequate. They reported a higher rate of decline in egg production with the suboptimal protein diets fed at the higher temperature, and concluded that, an inverse relationship existed between temperature and egg production at suboptimal protein levels.

The subject of strain and protein level interaction is a controversial one. Some investigators have reported positive interactions while others reported negative interactions.

Thornton and Whittet (1960), Aitken et al. (1973), Fernandez et al. (1973), and Hamilton (1978) respectively, reported no protein level by strain interactions.

But Sharpe and Morris (1965) and Sharpe et al. (1965) have reported protein level by strain interactions. Sharpe and Morris tested a strain of RIR X LS and a commercial hybrid of White Leghorn type and fed them diets of 16.5, 14.5, 12.5, and 10.5% protein and 2765 k cal ME/kg. A fifth diet contained 10.5% protein with 2165 k cal ME/kg. They reported that egg size was reduced by protein restriction in the heavy strain, but the light strain was not affected. They indicated that RIR X LS pullets showed no significant response in either egg number or egg weight to protein levels above 12.5% while the light hybrids showed a linear regression of rate of lay due to protein and showed the need for 16.5% protein in the diet.

Sharpe et al. (1965) tested the effect of changing dietary protein levels in two strains of pullets during the laying year from low to high and vice versa. They fed a "high" protein diet of 14%

crude protein with 2490 k cal ME/kg. and a "low" protein diet of 10.5% crude protein with 2420 k cal ME/kg. They found that the heavy strain layers produced eggs satisfactorily on both low and high protein diets and were not affected by changing from high to low, while the change from low to high stimulated an increased rate of lay above the level of that of the hens that received the high protein diet throughout the trial period. But, the low protein diet did not support egg production in the light hybrid hens.

Moreng et al. (1964) studied the effect of strain differences among four strains of commercial egg type birds on 13, 15, and 17% protein levels. The four lines were arbitrarily designated A, B, C, and D. Egg production results indicated that strain C produced significantly more eggs at 17% than at 13% with no difference between 13 and 15%, and 15 and 17%, respectively. Strains B and D showed no significant difference at the three treatment protein levels. Strain A produced significantly less eggs at 13% compared to 15 and 17% respectively.

Similarly, Deaton and Quisenberry (1965) reported a highly significant strain X protein level interaction for body weight, egg production, egg weight, feed efficiency, and Haugh unit value for four commercial strains of White Leghorn birds on diets with 14, 15, and 17% Protein levels.

Harms and Waldroup (1962) tested two strains of layers from the same breeder which were composed of C and A line strains. The birds were fed 11, 13, and 17% protein diets, and the results indicated that

the C strain had protein requirements in excess of 13% while that level adequately supported egg production of strain A layers.

Harms et al. (1966) studied strain or breed X protein level interactions with four strains of commercial egg type and a group of New Hampshire layers fed 17, 15, 13, and 11% protein corn-soybean diets. Their findings agreed with those of Sharpe and Morris (1965), whereby, the heavy breed laid more eggs on the 11% protein diet. This diet was not adequate for the light commercial egg type strains.

Balloun and Speers (1969) conducted three experiments with two commercial and three experimental strains of egg laying type birds and indicated significant differences among the strains in protein requirements. With Hylines, they found that a 16% protein diet supported satisfactory egg production with adequate body maintenance at peak production and feed conversion efficiency. The larger Ames incross birds needed a 14% protein diet for satisfactory egg production.

The three experimental Leghorn strains differed greatly in their protein requirements. The small size strain required less than 10 g protein intake for maximum egg production. The medium size and the large size required 18 g protein intake.

Finally, Pilbrow and Morris (1974) indicated that genetic differences in protein requirements may arise from one of two major sources. The first may be due to body weight maintenance and rate of production, and the second way may be due to efficiency in digestion and absorption of amino acids. Thus, one breed or strain of breed may

require more protein than another for each unit of body weight maintenance and egg synthesized.

IV. INFLUENCE OF FREE PLASMA AMINO ACIDS ON EGG PRODUCTION.

Several workers, Richardson et al. (1953); Hill and Olsen (1953); Charkey et al. (1953); Hill et al. (1961); and Zimmerman and Scott (1965); found a relationship between the amount of free plasma amino acids and the amino acid composition of the diet. When dietary lysine was increased from 0.5% to 1.9%, the plasma lysine concentration also increased progressively from 2.5 mg. to 10.5 mg./100 ml. Hill and Olsen (1963) and Zimmerman and Scott (1965) fed growing chicks graded levels of either lysine or valine over a wide range of levels above and below the normal requirement for maximum growth. Their results showed that accumulation of lysine occurred even when the dietary levels were below the normal level, but valine did not accumulate in the plasma when dietary levels were suboptimal. Richardson et al. (1953) studied the influence of amino acid supplements and protein on free plasma amino acid concentration levels and found that a relationship existed between dietary content of amino acids and protein on one hand, and plasma amino acids on the other. However, they noted that there was no apparent association between growth rate and plasma amino acid concentration.

The amount of normal plasma amino acid pool is usually very small; and therefore, its usefulness in measuring changes in plasma amino acid concentration is dependent upon the extent to which they reflect changes in other tissues of the body. Thus, Richardson et al. (1965) reported that lysine deficiency could reflect decreased plasma,

liver and muscle lysine levels, and an increased threonine level in these tissues and decreased arginine levels in plasma and muscle. In addition, they indicated that aspartic acid had the highest concentration in the liver, and alanine had the highest concentration in the muscle, respectively. Their observations gave rise to the suggestion that alanine was a major carrier of amino nitrogen degraded from non-essential and branched amino acids in the muscles to the liver.

Taylor et al. (1970) reported changes in plasma concentrations of free amino acids in relation to egg production in laying hens and indicated the following observations:

1. Amino acid concentrations are invariably higher during the night than day time.
2. Egg formation process is generally associated with an increase in non-essential amino acid concentrations with cystine and glutamic acids behaving like the essential amino acids.
3. Voluntary food consumption during egg formation process may be influenced by changes in concentration of essential amino acid levels in plasma. Arginine might be the possible amino acid influencing such voluntary food consumption changes.

Taylor et al. (1970), in the final conclusion of their investigation, suggested that free plasma amino acids in laying hens might not provide an adequate measure for the amino acid requirements of the laying hen nor the nutritive value of a particular protein or proteins for egg production. But Bray (1970) in his investigation of leucine

imbalance in laying hen diets showed a positive correlation between dietary leucine and plasma leucine, isoleucine, and valine levels.

V. AMINO ACID REQUIREMENTS FOR EGG LAYING HENS

According to Sunde (1972), amino acids are the most important units in proteins. It is a known fact that the quality of a protein depends upon its amino acid composition, balance, and availability. Factors such as environmental temperature, age of the bird, protein and energy level of the diet, and the stage of production are known to influence the dietary amino acid requirements of the laying hen (Britzman, 1964; Novacek, 1970; Choudhury, 1972; and Kashani, 1975). Amino acid requirements for monogastric animals are often expressed either as a percentage of the total diet or as a percentage of protein in the diet. If expressed as a percentage of the protein in the diet, then the amino acid requirement will drop as the level of the protein is increased above the level required to supply adequate amounts of other amino acids. However, when the percentage of dietary protein is increased, the minimum levels of many essential amino acids also appear to increase when expressed as a percentage of the diet. Combs (1969) attributed this phenomenon to the effect of a reduced protein level in-creasing voluntary food intake.

A. ESSENTIAL AND NON-ESSENTIAL AMINO ACIDS:

Amino acid requirements generally involve the balance between essential and non-essential amino acids. Wilcox and Hopkins (1906) and Osborne and Mendel (1914) reported that certain amino acids were essential in proper nutrition. Rose (1938) defined essential amino acids as "those which cannot be synthesized by the animal organism out of materials ordinarily available at a speed commensurate with the demand

for normal growth." Earlier studies to determine the essentiality of amino acids included feeding of a single purified protein known to be devoid or low in one or more amino acids (Rose, 1938), or substituting synthetic mixtures of amino acids in place of proteins, (Abderhalden 1912; Hegsted 1944; Grau and Almquist, 1944; and Almquist and Grau, 1944).

The successful determination of amino acid requirements by Mitchell (1950) utilizing carcass analysis to determine growth rate led to the investigation of possible amino acid requirements for egg production. Consequently, Johnson and Fisher (1956) determined the requirements of essential amino acids for egg production. They reported in their study that with the exception of glycine, all the amino acids essential for chick growth were also essential for egg production. The omission of either lysine, arginine, histidine, methionine, leucine, isoleucine, valine, threonine, tryptophan, and phenylalanine resulted in a prompt drop in feed consumption and pause in egg production. Further, they also observed that while the omission of glutamic acid did not affect feed consumption, normal egg production could not proceed in its absence.

In further investigations Johnson and Fisher (1958) determined the minimum requirements of the essential amino acids for the laying hen. They used the lysine content in whole egg protein as the basis for the ratio to the rest of the amino acids. They fed such calculated amino acid levels to laying hens and compared them with the same levels increased by 10% and found that the lower levels maintained a high rate

of egg production. Johnson and Fisher (1959) again attempted to relate their data obtained with synthetic amino acids to practical rations and indicated that practical diets with 10 to 11% protein maintained a high rate of egg production over a prolonged period. Later workers such as Bray (1960) and Fitzsimmons et al. (1963) collaborated those findings.

B. AMINO ACID BALANCE:

Amino acid balance is the relative proportional presence of all amino acids in a given diet. Therefore, the absolute quality of a given protein or nutritive value of a given diet depends on its amino acid balance. Thus, any deviation from the optimum balance of amino acids for body weight maintenance and egg production will ultimately result in less efficient utilization of protein, since the requirement of one amino acid is affected by the relative content of the other; and hence, the interrelationship or interactions among amino acids needs to be determined. D'Mello and Lewis (1957c) indicated that diets should contain a satisfactory amino acid balance in order to determine the chicks' requirements for the essential amino acids.

Few studies have been reported on the determination of the optimum ratio between essential and non-essential amino acids for maximum protein utilization. Sugahara and Aryoshi (1968) reported a ratio of 1:1 for optimum feed efficiency in layers and 1.5:1 for maximum growth in chicks. Shapiro (1968) indicated that the ratio could vary from 0.8 to 1.2 in laying hens for peak nitrogen retention. Manoukas and Young (1969) and Reid et al. (1972) suggested that the ratio of essential amino acids to non-essential amino acids could vary from 0.7 to 1.15

without any adverse effect on egg production performance of the hen, provided the hens consumed 14 to 15 g of protein daily.

A study of the comparison between essential and non-essential amino acid ratio requirements for layers and chicks indicated that hens, by and large, require relatively lower ratios than chicks; and the data agree with the suggestion of Manoukas and Young (1969) that non-essential amino acids are utilized for body functions other than synthesis of egg protein during egg production.

C. AMINO ACID ANTAGONISM:

1. Lysine-Arginine:

Antagonism is a hostile relationship which exists between two amino acids, whereby the excess of one requires corresponding increased amounts of the other. Thus, antagonistic relationships between lysine and arginine, whereby excess lysine necessitated higher arginine requirements, have been reported by several workers (Snetsinger and Scott, 1961; O'Dell et al., 1962; Jones 1964; O'Dell and Savage 1966; Boorman and Fisher, 1966; Smith and Lewis, 1966; Jones et al. 1967; and D'Mello and Lewis, 1970a). Wilburn and Fuller (1975) reported that the addition of lysine to a corn-soy diet up to a level to that in a casein diet increased the arginine requirement of broilers from 1.53 to 1.73%. Savage (1972) observed accentuated arginine deficiency symptoms when lysine was incorporated as a major source of protein in a casein diet. The arginine content of casein is approximately one-half that of lysine. Fisher et al. (1960) and Dobson et al. (1964) showed that the

high lysine content of casein is the principal cause of high arginine requirements of chicks on a casein diet.

The mechanism causing the lysine-arginine antagonism has been extensively studied. Austic and Nesheim (1970) reported that arginine metabolism occurs mainly through the arginase pathway. Jones et al. (1967) indicated that an excess of dietary lysine (.2%) elevated the kidney arginase activity in chicks to five times their normal level and further observed that plasma arginine dropped 50% below the control in twelve days.

Nesheim (1968) and Austic and Nesheim (1970) also reported induction of kidney arginase activity through increased dietary lysine levels.

However, lysine and arginine antagonism is of relatively insignificant concern in practical laying diets since such diets are generally deficient or marginal in lysine and adequate in arginine. But this antagonism is of concern, and nutritionists should be well aware of it and guard against it.

2. Leucine, isoleucine, and valine interrelationships:

D'Mello and Lewis (1970b) showed that excess leucine retarded the growth of chicks fed diets marginal in valine and isoleucine, and that additions of valine and isoleucine corrected the situation. In a further report, D'Mello and Lewis (1970c) indicated that the chick's dietary requirements for valine and isoleucine increase with an increase in leucine.

D'Mello and Lewis (1971) conducted a paired feeding growth trial of chicks and reported that an intake of excess leucine lowered the free plasma valine and isoleucine concentrations. Tuttle and Balloun (1976) carried out two studies with turkey poults and indicated that excess leucine reduced plasma levels of both valine and isoleucine, and valine alone in another trial. Based on those findings, they concluded that interactions of leucine-valine, leucine-isoleucine, and valine-isoleucine existed for the poult. Bray (1970) reported interactions among leucine, isoleucine, and valine in a laying pullets study. Deletion of 0.3% DL-isoleucine, from a low protein diet (8.5%) which was supplemented with the limiting amino acids, resulted in 25.7 and 55.7% depressions in egg production when imbalanced; and leucine balanced diets were used, respectively. Valine was reported to be the second limiting amino acid in that study where 85% of the protein was supplied by corn. In another trial Bray (1970) reported egg production to be maximum when both isoleucine and valine were added to the diet.

Muller and Balloun (1973) indicated that the detrimental effect of L-Leucine (0.75%) in a 12% protein diet could be reversed when both L-isoleucine (0.175%) and DL-valine (0.35%) were added to the diet, and that addition of isoleucine (0.35%) alone caused further reduction in egg production.

In a later study, Muller and Balloun (1976) showed that a low protein diet (10%) was imbalanced by a 0.75% L-leucine addition, while a high protein diet (19%) was not affected by egg production and feed

consumption data. They indicated that with the 10% protein diet, the excess leucine could not be reversed with 0.1% DL-isoleucine addition.

3. Amino acid sparing effects:

Sulfur containing amino acids--methionine and cystine. (Methionine-choline and methionine-threonine interrelationships). Methionine can adequately furnish the sulfur amino acid requirements for laying hens. According to Graber and Baker (1971), it is most economical to supplement poultry diets marginal in sulfur amino acids with methionine but also cautioned that consideration should be given to other sulfur amino acids and inorganic sulfates.

No dietary requirement has been determined for cystine, which is the oxidation product of cysteine. Cystine is added to diets in order to supply the need for cysteine. It has been known that cystine can furnish about 50% of the sulfur amino acid requirements in growing chicks (Graber et al., 1971; Sasse and Baker, 1974a; and Soares 1974). Graber and Baker (1971) indicated that the requirement for sulfur-binding amino acids was lower when a combination of methionine and cystine was used than when methionine alone was used. Baker (1976) reported that methionine is about 80% efficient in being converted to cystine, based on molecular weight conversion (149 vs. 121).

While methionine can spare the dietary requirements for cystine, the latter cannot be converted to methionine (Role and Rice, 1939); and also methionine can spare the needs of choline and threonine; but they, too, cannot be converted to methionine.

Finally, it has been shown that about 15% of the cystine dietary requirements for the chick may be furnished by sodium or potassium sulfate (Sasse and Baker, 1974a,b; and Soares, 1974).

4. Phenylalanine-tyrosine interrelationship:

Almquist and Grau (1944) reported that a high phenylalanine level eliminates or spares the need for tyrosine. But Grau (1947) indicated that while phenylalanine can be converted to tyrosine, the latter cannot be converted to phenylalanine and that only phenylalanine alone can satisfy its dietary requirements. The situation is similar to the interrelation existing between methionine and cystine. Further, Grau (1947) showed that the conversion of phenylalanine to tyrosine was efficient. Sasse and Baker (1972) confirmed Grau's data and showed that the total aromatic amino acid requirement was not higher when phenylalanine alone was fed than when a proper combination of phenylalanine and tyrosine was fed.

However, Johnson and Fisher (1956) indicated that laying hens converted phenylalanine to tyrosine with difficulty and inefficiently.

5. Serine-glycine interrelationship:

The following workers have reported that serine can completely satisfy the glycine dietary need for chicks' growth (Akraabawi and Kratzer, 1968; Baker et al. (1968); and Rabbani et al. 1973). Nog and Coon (1976) indicated that feeding optimum levels of glycine to chicks during the first nine days of life reduced the need for glycine and serine during their remaining 21-day feeding period. Johnson and Fisher (1956), in their historic determination of essential amino acids

for the hen, did not classify serine nor glycine as an essential amino acid. So far, there is no evidence to show that deficiencies of these two amino acids in the diet could affect maximum egg production. However, Baker et al. (1968, 1972) and Featherston (1975) have shown data that deletion of glycine and serine in broiler diets depressed growth rate by 20 to 25% in the second week of life.

6. Arginine-proline- glutamic acid interrelationships:

Arginine, proline, and glutamic acid have a common pathway of metabolism; though proline is a non-essential amino acid and can be synthesized by the chick. Nevertheless, a proline deficiency can effect a 10-20% growth retardation and reduced feed efficiency (Roy and Bird, 1959; Greene et al. 1962; Adkins et al. 1962; Bhargava et al. 1971b; and Austic 1973). Austic (1973) reported from a study based on C¹⁴-labeling that 7% of the arginine was directly converted to proline when the diet was lacking in proline and was only adequate in arginine for chicks. Further, Austic (1973, 1976) indicated that arginine is preferably converted to glutamic acid.

Maruyama et al. (1976a), in a study using only essential amino acids in chick diets, reported that glutamic acid was more effective in promoting chick growth than any other non-essential amino acid; although Johnson and Fisher (1956) did not classify glutamic acid as an essential amino acid. It was significant to note that the deletion of glutamic acid in their studies resulted in a drop in egg production with no measurable effect on feed consumption.

VI. ABSORPTION OF AMINO ACIDS

Lerner (1971), Hudson et al. (1971), and Kan (1975) reviewed the intestinal absorption of amino acids and peptides in domestic fowl and observed the following problems associated with absorption of amino acids in general:

1. The active transportation of amino acids and peptides against concentration gradients from the intestinal lumen to the blood.
2. Occurrence of interaction during absorption of amino acid mixtures resulting either in stimulatory or inhibitory effects on absorption as compared to only a stimulatory effect where only a single amino acid is transported and absorbed.
3. Presence of sugars which may inhibit amino acid transportation.
4. The animals own physical and physiological conditions which may be influenced by age. Further, dietary deficiencies such as lack of vitamins and essential fatty acids; and presence of antibiotics, or restricted feeding, may have an effect on intestinal amino acid absorption.

Several workers have reported that amino acids are actively transported. The first reports came from Gibson and Wiseman (1951). However, Kratzer (1944) had earlier reported that amino acids were transported by diffusion, although his data might have been due to the very high concentrations he used in his studies. Wiseman and Gibson (1951) and Tasaki and Takahashi (1966), the former in a study with rats and the latter in a study with chickens, respectively, provided

conclusive data to show that amino acids are actively transported. Further, Tasaki and Takahashi (1966) and Scharrer (1971) compared amino acid transportation in rats and chickens and reported identical results except for lysine and histidine. The highest rate of absorption was for the neutral amino acids, followed by the basic amino acids, and lastly the acidic amino acids. Wiseman and Gibson (1951) had earlier conclusively reported that the L-form of amino acids are absorbed faster than the D-form of amino acids. Many other workers have reported on the active transportation and absorption of the L-form of amino acids in chickens (Perry et al., 1956; Fearon and Bird, 1967; Holdsworth and Wilson, 1967; Nelson and Lerner, 1970; Wakita et al., 1970; Yokota and Tasaki, 1970; Herzberg et al., 1971; Scharrer, 1971; Burrill and Lerner, 1972; Sherrin and Bird, 1972; and Kushak et al., 1973)

Lerner and Taylor (1967), Herzberg et al. (1971), and Burrill and Lerner (1972) showed that the D-amino acids usually inhibit the transportation of the L-amino acids, but they also observed that in some instances the effect was stimulatory. Further, they also observed that amino acids handled by the same transport system usually inhibit each other's transportation while preincubation with amino acids often stimulates the transportation of other amino acids via an exchange diffusion. Amino acids handled by different transport systems will either stimulate each others transportation or not, for the results are not always reciprocal. These findings agreed with several studies reported in chickens by Paine et al. (1959), Hudson (1969), Herberg et al. (1971), LaBelle et al. (1971), Lerner and Burrill (1971), Burrill and

Lerner (1972), Lazarov and Alexandrov (1972), Lerner and Miller (1972), Herberg and Lerner (1973), Kushak et al. (1973), Miller et al. (1973), and Tucker and Kimmich (1973).

Fearon and Bird (1967), Teckell et al. (1967), Hudson (1969), Wakita et al. (1970), Yokota and Tasaki (1970), and Scharrer (1971) reported that the ileum is the site for maximum amino acid absorption in the chicken and that no absorption of amino acids takes place in the duodenum, colon-rectum, or cecum. However, they indicated that the embryonic cecum and colon can actively absorb amino acids.

Herberg et al. (1971), Lerner and Burrill (1971), and Miller et al. (1973) reported that amino acid transportation is dependent on sodium concentration and that exclusion of sodium from the external medium will result in great inhibition of amino acid transportation. Further, they also observed that transportation of basic amino acids is less sensitive to sodium concentration, the acidic amino acids are the most sensitive, and the neutral amino acids lie in between or are intermediate in sensitivity.

Klan (1975), in his review, stated that generally the non-metabolizable but actively transported sugars inhibit transportation of amino acids while the metabolizable sugars that are actively transported stimulate amino acid transportation. Further, Klan (1975) reported that vitamins B₆, D, E, and B₁ deficiencies lower amino acid absorption and that vitamin C has no effect. However, Chez et al. (1969) fed rabbits with vitamin B₆ deficient diets and found no effect on amino acid absorption rate. However, Tasaki and Yokota (1970) studied the effect

of deoxy pyridoxine in chickens and indicated that under certain conditions, vitamin B6 deficiency inhibits amino acid absorption.

Antibiotics such as oligomycin, cycloheximide and actinomycin, which directly inhibit energy production and protein synthesis, and digitalis-like compounds which inhibit the sodium pump, also inhibit amino acid absorption. Surgical operations, such as hypophysectomy and adrenalectomy, also inhibit amino acid absorption. But Draper (1958) showed that penicillin enhances lysine absorption. Lotenkov and Podluznaya (1967) reported that chlortetracycline stimulates the absorption of L-arginine, DL-valine, L-histidine, DL-leucine, DL-lysine, DL-methionine, DL-phenylalanine, and DL-threonine. Kimmich (1970) reported that oligomycin inhibits L-valine absorption in isolated intestinal epithelial cells. Lazarov and Alexandrov (1973) indicated that testosterone-propionate or progesterone reduced the absorption of ^{35}S -methionine and ^{35}S -cystine from the duodenum of 4-6 week old chicks. Preston-Mafham and Sykes (1970) reported that Eimeria acervulina infection resulted in a decreased absorption of L-histidine both in vivo and in vitro, with the maximum effect being reached on day 5 post infection.

There have been some reports on the influence of metabolic inhibitors and anoxia on intestinal amino acid transportation and absorption. Since scientific evidence has shown that amino acids are actively transported, it is only logical to believe intuitively that metabolic inhibitors and anoxia which inhibit aerobic energy generation might equally affect amino acid transportation. However, the direct

source of such energy is not known; but the speculation is that ATP would be the likely source. However, Bronk (1972) showed that when the plasma ATP concentration falls to 10% of the normal content, active transport still exists. Reiser and Christiansen (1971) found with isolated intestinal epithelial cells of rats that ATP hindered amino acid absorption; but ADP, 5'-AMP, cyclic 3', 5'-AMP, GTP, and inorganic phosphate did not. However, Kinzie et al. (1973) reported a contrary view that ATP and AMP have no influence on leucine absorption in rat's jejunum, but observed that dibutyl cyclic AMP or cyclic 3', 5'-AMP together with theophylline enhance absorption. Further, they also indicated the latter substance had no stimulatory effect on leucine absorption in the ileum of the rat.

Many reports have indicated that peptides are also absorbed in the small intestine. The peptides could be absorbed in the intestine and be hydrolyzed either superficially or intracellularly, and the free amino acids circulate in the blood. But some peptides such as glycylglycine and hydroxy prolylproline are very resistant to hydrolysis; and so, part of them may appear in blood uncleaved. Several workers have reported that peptides are absorbed faster than amino acids. Such evidence was shown by Kushak et al. (1973) with chickens in a study of glycine uptake from the peptide glycylleucine. Crampton et al. (1971) showed similar results in a study with rats. They indicated that a tryptic hydrolysate of several proteins which contained a peptide with 2-6 amino acids was absorbed faster than an amino acid mixture.

Age, dietary, and disease conditions are known to influence the absorption of amino acids. Penzes (1972) reported such an influence in several studies he carried out with rats which involved changes in amino acids throughout a lifetime. Restricted feeding enhanced amino acid absorption, and the same is also true for high protein levels, 70-80% of the diet. Further, marked differences do exist in chickens between embryonic time around hatching and post embryonic hatching time as related to rates of amino acid absorption. Such studies were reported by Holdsworth and Wilson (1967), Hudson (1969), Pratt and Turner (1971) respectively. Lotenkow and Podluznaya (1967) indicated a decrease in amino acid absorption during the first 1-75 days of life. Lazarov and Alexandrov (1972) withheld feed but allowed water ad libitum and observed a decrease in methionine absorption from the duodenum, the minimum being reached after 15 days of feed withdrawal.

It would appear from the data indicated that amino acids and peptides have separate transport systems. This suggestion is supported by the seeming spatial difference in maximum absorption and the non-interaction between amino acid and peptides absorption. The idea is further supported by the incidence of two human diseases, Hartnup disease and cystinuria, in which the absorption of certain free amino acids is negligible in the intestine, while the peptides containing the same amino acids are normally absorbed. Addison et al. (1972, 1973) showed evidence of active transport of slowly hydrolysed peptides in the jejunum of a hamster and an interaction between the peptides during absorption as well.

Reports have been made by some investigators that amino acids are absorbed at different rates. Adibi et al. (1967 a,b) reported in their study of amino acid absorption from the small intestine of young men that methionine, isoleucine, and leucine were absorbed rapidly. The rates of proline, arginine, alanine, and phenylalanine were much slower. They further observed that glutamic and aspartic amino acids (dicarboxylic acids) were either absorbed more slowly or, perhaps, were replaced by the alpha keto acids in the process of transamination. Tasaki and Takahashi (1966), in their study with the domestic fowl, reported that the rates of absorption of arginine, tyrosine, aspartic and glutamic acid, and glycine were relatively slow compared to the rest of the amino acids.

In summary, the subject of amino acid absorption in the intestine is not yet totally clarified. There is still some discrepancy in the literature. There is no agreement that all the amino acids absorbed in the intestine appear in the blood. Jacobs and Largis (1969) observed the presence of free amino acids from dietary sources in the lymph. Secondly, the uptake of amino acids in the intestine may not be a one-way traffic; it is likely that some may flux back to the intestinal lumen as reported by Schedl et al. (1969). Thirdly, the transport agent's nature is still in doubt, even though Reiser and Christiansen (1968) found a heptane soluble fraction of the brush border which binds to amino acids.

VII. THE EFFECT OF DIETARY AMINO ACID COMPOSITION ON

EGG ALBUMEN AMINO ACID PROFILE

Egg protein has been acclaimed to be of the highest biological value in human nutrition. It serves as a relative index to evaluate the biological value of other proteins. Since the egg derives its protein from dietary sources, it is of importance to know the effect of dietary protein on egg protein composition.

Csonka et al. (1947) reported a significant increase in both methionine and cystine contents of egg albumen when dietary protein was increased with casein supplementation. However, Ingram et al. (1950) indicated no difference in concentrations of lysine, tryptophan, methionine, and cystine in egg albumen when laying hens were fed with diets deficient in those amino acids. Similar results were reported by Ingram et al. (1951) and Milligan et al. (1951). Milligan et al. (1951) fed a 9% protein diet and found no difference in lysine and methionine contents of the egg albumen. Choudhury (1972), however, reported an influence of dietary amino acid composition on egg albumen. He reported an increase in amino acid concentration of egg albumen due to feeding higher dietary protein levels or by supplementing low protein diets with 0.1% DL-threonine or 0.5% valine. In contrast, Kashani (1975) did not find consistent changes in amino acid composition of egg albumen when methionine, lysine, tryptophan, and isoleucine were added to a 12% protein diet.

VIII. NUTRITIVE VALUE OF SOYBEANS AND SOYBEAN MEAL IN POULTRY AND OTHER FARM ANIMALS

A. POULTRY:

1. Egg Laying Performance in Hens:

The nutritive value of raw soybean meal and heat treated soybean meal has been evaluated in comparison studies with laying hens. Carver et al. (1946), Fisher et al. (1957), Griminger and Fisher (1960), and Saxena et al. (1963a,b) reported comparable and satisfactory egg production performance of hens fed raw soybean meal and heat treated soybean meal diets. Carver et al. (1946) fed a 13% crude protein level of extracted raw soybean meal and heat treated extracted soybean meal, while Fisher et al. (1957) fed a semipurified raw extracted soybean meal supplemented with methionine and vitamin B₁₂ at a 12.21% crude protein level. Also, Saxena et al. (1963a,b) supplemented a raw soybean meal diet with methionine, whereas Griminger and Fisher (1960) fed raw unextracted soybean meal as the major source of protein.

On the contrary, Renner and Hill (1960), Hill and Renner (1963), and Rogler et al. (1964) indicated that egg production performance of hens fed a raw soybean meal diet was poor compared to that with a heat treated soybean meal diet.

Rogler et al. (1964), following the contradiction in the earlier reports on comparable satisfactory egg production performance by hens on a raw soybean diet or a heat treated soybean diet, conducted two experiments to re-evaluate the reports. They fed diets containing raw soybean meal as a 100% replacement of commercial soybean meal to

study the effect of both diets on egg production, feed efficiency, pancreatic hypertrophy, and fatty acid content of tissue and egg yolk lipids. The following findings were reported:

I. Egg production and feed efficiency were reduced in the birds fed raw soybean meal diet and pancreatic hypertrophy was produced.

II. Heat treated unextracted soybean meal fed hens were equal to commercial soybean meal fed hens in egg production and feed efficiency.

III. Hens fed a heat treated unextracted soybeans diet produced an increase in linoleic and linolenic acids in the thigh and egg yolk lipids at the expense of palmitic, palmitoleic, and oleic acids.

These findings as to egg production agreed with those of Renner and Hill (1960), Hill and Renner (1963), and with later reports of Nitsan (1965), Nesheim and Garlich (1966), and Lepkovsky et al. (1965). The latter reported poor digestibility of raw soybean meal compared to heat treated soybean meal.

2. Unidentified Growth Factor:

Numerous reports have been published on studies of an unidentified growth factor in soybean meal protein for stimulating growth in chick and turkey poults (Hill, 1948; Hill and Briggs, 1950; Savage et al. 1950; Patrick, 1953; Kratzer et al. 1958, 1959; Vohra et al. 1959; Wilcox et al. 1961a,b; Westerfield and Harman, 1962; Carlson et al. 1963a,b; Griffith and Young, 1964; Kratzer et al. 1964; and Jensen and Mraz, 1966).

Hill (1948) showed evidence of variations in unidentified growth factor activities in soybean meal due to different origins.

Kratzer et al. (1958, 1959) indicated that a methanol extract of soybean meal greatly improved growth rate when added to an isolated soybean protein-glucose type ration.

Wilcox et al. (1961a) reported that a water extract of soybean meal added to the type of diet used by Kratzer et al. (1958, 1959) improved growth rate in turkey poults. Wilcox et al. (1961b) indicated that both a dialyzable (inorganic factor) and a non-dialyzable (organic factor) fraction of a water extract of soybean meal equally stimulated growth of turkey poults.

Carlson et al. (1963a, b) reported a growth stimulating effect of water extract of soybean meal when added to an isolated soybean protein diet, but it only partially remedied the rachitogenic effect of isolated soybean protein.

Griffith and Young (1964), Kratzer et al. (1964), and Jenson and Mraz (1966); all indicated that the unidentified growth, the rachitogenic, and the calcification effect factors in soybean meal protein were different factors.

Scott et al. (1962) suggested the presence of an unknown factor in soybean meal which increased the phosphorus availability of anhydrous dicalcium phosphate for turkey poults that were fed isolated soybean protein.

B. SWINE:

Many investigators studied the nutritional value of raw soybeans for growing-finishing swine and indicated that it was a poor protein supplement when fed as the only source of protein. In corn-based diets raw soybeans were detrimental because of their soft pork effect and growth depression effect, respectively, (Weaver, 1929; Robinson, 1930a; Bull et al., 1931; Helser et al., 1939; Hostetler and Halverson, 1940; Jimenez et al., 1963; and Combs et al., 1967).

Wahlstrom et al. (1971) investigated the effect of infra-red cooked soybeans for growing-finishing swine following an earlier report of Featherston and Rogler (1966) that an infra-red cooked soybean protein supplement supported maximum growth in chicks. Wahlstrom et al. (1971) utilized 72 crossbred pigs in their experiment and fed diets with the protein supplement consisting of only soybean meal, 50% soybean meal plus 50% cooked beans, cooked whole beans, and finally cooked beans supplemented with 0.1% L-lysine and 0.25% DL-methionine. The pigs were fed from an average weight of 29.5 kg. to a finishing weight of 93 kg., and they reported the following results:

1. Rate of daily body weight gain and feed efficiency of pigs fed cooked soybean diets were either equal to or superior to those fed soybean meal diets.

2. Pigs on cooked soybean diets consumed less feed.

3. The dressing percentages of the pigs on the cooked soybean diet were significantly higher.

4. Pigs fed cooked soybean diets had higher back fat thickness.

5. Pigs fed cooked soybeans had less firm carcasses with more linoleic acid resulting in higher iodine numbers.

6. Pork acceptability was not affected as evaluated by a taste panel.

The findings of Wahlstrom et al. (1971) agreed with those of Romans et al. (1970) who had fed cooked soybeans and found the same chemical characteristics of pork carcass fat with higher iodine numbers.

Combs et al. (1967) made a comparison of the effect of raw and heated soybeans on gain, nutrient digestibility, plasma amino acids, and other blood constituents of growing-finishing swine and reported the following results:

1. Coefficients of digestion for dry matter, ether extract, and plasma contents of urea, nitrogen, cholesterol, calcium, and phosphorus were similar for both treatments.

2. Heat treatment improved body weight gain and protein digestibility.

3. Protein and calorie supplementations were not effective in overcoming the growth depression by raw soybeans.

Similar beneficial effects of heat treated soybean meals over raw soybean diets were reported by Becker et al. (1953) and Nickelson (1960). However, Nickelson et al. (1960) indicated that there was no statistically significant difference between carcass quality of pigs fed raw soybeans and heat treated soybean meals.

Soybean flour has been fed to early weaned pigs in milk replacer diets. Improvements with enzyme supplementation on such diets have been reported (Hays et al. 1959; Combs et al. 1963; Meade, 1967; Lewis et al. 1955). But Cunningham and Brisson (1957), Combs et al. (1960), and Maner et al. (1961) reported a contrary view that there was no improvement with enzyme supplementation.

However, Lennon et al. (1971), following reports by Colvin and Ramsey (1968, 1969) of success in using a alkali or acid treated soyflour in milk replacer diet for calves, duplicated the idea for early weaned pigs. The alkali treated diets were subjected to a pH of 10.6 for either 30 minutes or five hours prior to incorporation in the diets. The following results were reported:

1. Pigs on the soyflour alkali treated diet grew faster and had better feed efficiency than the untreated group.

2. There was no difference between either 30 minutes or five hours duration of exposure to an alkali medium.

3. Marked improvement was also shown when ground soybean meal was treated with alkali.

4. Acid treated soyflour did not improve performance.

5. Supplementation with methionine, threonine, and lysine did not elicit any improvement.

6. Analysis of the alkali treated diet and the untreated one did not show any difference in amino acid content.

Lennon et al. (1971), like Colvin and Ramsey (1968, 1969), could not explain any scientific reasons responsible for the favorable

changes brought about by the alkali. However, contrary to Lennon et al. (1971); Colvin and Ramsey (1968) had equally favorable results with either alkali or acid treated soyflour as a milk replacer diet for calves. However, Crenshaw and Danielson (1985) studied the nutritional effect of raw soybeans for gestating and lactating sows. They carried out three experiments on sixty crossbred gilts through three parities and fed them with raw soybeans and regular soybean meal diets, respectively. Diets were fed at a 14% crude protein level up to 110 days of gestation, and all treatment groups were switched to a 15% protein lactation diet with regular soybean meal. All diets were corn based. The following findings were reported:

1. No difference in the number of pigs farrowed alive between the two treatments.
2. No difference in average number of pigs weaned.
3. An increase in birth weight for the raw soybean diet ($P < 0.05$), but there was no difference in weaning weights.
4. No difference in feed consumption during lactation.
5. No difference in sow's weight during gestation or lactation between the two treatments.
6. An increase in milk fat at day-3 of lactation for the raw soybean diet.
7. The authors concluded from their data that raw soybeans could replace soybean meal diets for gestation.

B. RUMINANTS:

Soybean protein has been fed to ruminants as an acid or alkali treated soyflour (Colvin and Ramsey, 1968, 1969), as heat treated soybean meal (Glimp et al., 1967; Hudson et al., 1964), and as soybean meal (Plegge et al., 1982).

Colvin and Ramsey (1968) studied the nutritive value of acid treated soyflour protein in a milk replacer for nursing calves. Fully cooked ground soyflour was treated with acid and fed as the only source of protein, and the results indicated greatly improved performance of the calves in rate of gain.

Thus, Colvin and Ramsey (1969), being encouraged by their earlier data of acid treatment, investigated the effect of alkaline treatment on soyflour protein in a milk replacer for calves. They obtained results similar to their earlier study. The calves grew as much as those on acid treated soyflour. Both experimental data indicated that calves fed the acid and alkaline treated soyflour grew twice as fast as the untreated controls.

Colvin and Ramsey (1969) investigated the evidence for the apparent better performance of the acid and alkaline treated soyflour fed calves over the controls by comparing the rate of passage between the treated soyflour diets and whole milk which was the control diet. They found that the rate of passage of dry matter and total nitrogen was more rapid for the treated soyflour diets than whole milk; and secondly, the pH declined more rapidly with whole milk after feeding than with the treated soyflour diet. But they could not find any statistical significance between the two rates of passage, and

therefore, could not give any scientific explanation as to why the calves fed the acid or alkaline treated soyflour diets performed better than the control group.

Hudson et al. (1969) conducted an experiment to evaluate the nutritive value of heat treated soybean meal protein on growth performance of growing lambs. Crossbred lambs weighing approximately 16 kg. were randomly allotted to three dietary treatments of 10, 12, and 14% heated soybean protein diets to determine rate of growth, digestibility, and nitrogen retention, compared to untreated control groups. The soybean meal was heated at 149°C for four hours. The following results were reported:

1. Daily weight gains increased significantly ($P < 0.05$) with increased protein levels which indicated that the crude protein requirement for growing lambs might be higher than the level reported by Laster (1964). There was no improvement on gains at 10 and 14% protein levels; but significant improvement occurred at the 12% protein level, which indicated that the 12% protein level might be near the marginal protein requirement level for lambs of that age. That suggestion would seem to collaborate with the findings of Cuthbertson and Chalmes (1950); which showed that the amount of ammonia produced is only a critical factor when suboptimal protein is fed and not where the protein level simply meets the animals requirements.

2. Rumen ammonia increased with increased protein ($P < 0.01$) and decreased with heated soybean level ($P < 0.05$) which agreed with the reports of Chalmers et al. (1954) and Sherrold and Tillman (1962).

3. Feed efficiency significantly improved with 12% over 10% protein but not over 14%, which once again indicated that the 12% protein level is close to the minimum level of protein required by early weaned lambs. Heating the soybean meal also improved feed efficiency ($P<0.05$) and again with 12% over 10% but not with 14% over 12%.

4. No difference in volatile fatty acids concentration was obtained, contrary to the report of Glimp *et al.* (1967), who indicated reduced levels of branched-chain fatty acids, and Stakcup and Davis (1966), who reported higher concentration of iso-valeric and n-valeric acid for feeding similarly heat treated soybean meal.

5. Cellulose digestibility improved significantly ($P<0.01$) with protein increased from 10% to 12% but not when it increased from 12% to 14%. Apparent digestibility of protein improved significantly ($P<0.01$) with increases in protein level, but the heat treatment slightly reduced protein digestibility. This agreed with the reports of Tillman and Kruse (1962). However, Sherrod and Tillman (1962), and Glimp *et al.* (1967) reported contrary data in which they showed an increase in nitrogen retention from heat treatment soybean meal.

6. Plasma urea levels increased with increased protein levels ($P<0.01$), but heat treatment of soybean meal had no effect.

7. Molar concentrations of acetic, iso-valeric and n-valeric acids were not affected by an increase in protein levels and neither heat treatment had any effect on these parameters. Propionate production decreased with increased protein levels ($P<0.01$) while butyrate increased ($P<0.01$). According to El-Sharzly, the proportion of butyric

acid production is an indication of the extent of degradation of ingested protein.

Plegge et al. (1982) reported increased utilization of soybean meal when roasted at 262.4°F or 291.2°F resulting in higher nitrogen retention.

C. DELETERIOUS EFFECTS IN RAW OR IMPROPERLY HEAT TREATED SOYBEAN MEAL DIETS:

1. Rachitogenic Effect:

Carlson et al. (1964a), in a four-week study on the growth stimulating effect of a water-extract of soybean meal discovered that isolated soybean protein produced rickets in Broad Breasted turkey poults. Carlson et al. (1964b) investigated the rachitogenic effect of soybean protein further to determine whether the property also existed in raw soybean protein. Both commercial soybean meal and raw soybean meal water-extracts were prepared and incorporated into diets calculated to contain all the necessary known ingredients for optimum poult growth. The overall results obtained in a series of six experiments indicated that raw soybean meal contains the same kind of rachitogenic effect, but the effects were not as severe as those shown with isolated soybean protein. Raw soybean meal also has an anti-ricketic effect which modulates its rachitogenic effect; and hence, its rachitogenic effect is not as pronounced as that shown by isolated soybean protein. Further highlights of the experiments indicated that the rachitogenic effect was only partially remedied by supplementation with heated or raw soybean meal or by water-extracts of raw or heated soybean meals,

but growth rate was markedly improved. Large amounts of vitamin D₃ supplementation only elicited suboptimal growth responses. Raw or heat treated soybean protein seemed to contain factors necessary for optimum growth rate and bone calcification even when the level of vitamin D₃ was greatly increased. However, Carlson *et al.* (1964b) did not find any beneficial effect from adding Ethylene Diamine Tetra Acetic Acid (EDTA) to the isolated soybean protein diet. This is somewhat contrary to the claim of Davis *et al.* (1962) that an EDTA addition to an isolated soybean protein diet remedied the condition of the unavailability of zinc, manganese, and copper, which are chelated by a compound present in isolated soybean protein.

Following the reports of Carlson *et al.* (1964a,b) Jensen and Mraz (1966) carried out a similar study with chicks to determine if chicks develop rickets in the same manner as seen in turkey poults. Secondly, they wanted to find out if the rachitogenic factor and the growth factor were one and the same or two different entities. Thirdly, they wanted to determine what influence the previous protein source and vitamin D₃ level had on radio-active absorption of calcium in intestinal loops. Their results indicated the following:

1. Chicks fed on 40% isolated soybean protein with graded levels of calcium, phosphorus, and vitamin D₃ much higher than the National Research Council (N.R.C.) (1977) recommendations showed reduced bone ash.

2. Supplementing the basal diet with commercial soybean meal or heat treating the isolated soybean protein or reducing the level of isolated soybean protein improved bone ash.

3. Water-extracts of soybean meal significantly improved growth rate but failed to improve bone ash.

Thus, Jensen and Mraz (1966) concluded that the growth stimulating factor and the rachitogenic factor were two separate or individual factors since the water-extract only improved growth rate and not bone ash. The Jensen and Mraz (1966) report confirmed the report of Griffith and Young (1964). The latter indicated evidence of two factors in soybean meal--a water-soluble fraction in the residue which improved growth rate and an insoluble fraction in the residue which improved the biological value of phosphorus from anhydrous dicalcium phosphate. Further, Kratzer et al. (1964) indicated that increased amounts of vitamin D_3 failed to alter the growth response of turkey poults fed diets supplemented with methanol extracts of soybean meal. Thus, they indicated that the growth factor and the calcification factor were two separate factors.

4. Calcium absorption from the intestinal loops of fasted chicks previously fed an isolated soybean protein diet was the same as that from chicks previously fed a casein-gelatin diet.

5. The authors concluded from their experimental data that, raw isolated soybean protein does not interfere with the apparent metabolism and absorption of vitamin D_3 but does interfere with normal absorption of either calcium or phosphorus or both. This confirmed an

earlier study on the uptake of vitamin D₃ and calcium in rachitic chicks by Wasserman (1962).

2. Growth Inhibitor, Anti-Tryptic or Anti-Proteolytic Factor:

The literature is full of numerous published reports on the growth inhibitory effect observed when raw soybean meal is fed to monogastric animals such as swine, poultry, and rats. These included for swine, Osborne and Mendel (1917), Weaver (1929), Robinson (1930a), Jimenez et al. (1963), and Combs et al. (1967); for poultry, Ham et al. (1945), Evans and McGinnis (1946), Clandinin et al. (1947), Brambila et al. (1961) and Saxena et al. (1961); and for rats, Morgan and King (1926), Johnson et al. (1939), Booth et al. (1961), and Hayward et al. (1963).

The consensus of opinion by all investigators engaged in studies of the growth depression effect of raw soybean meal is that it contains an active principle which interferes with normal protein metabolism in young monogastric animals, resulting in retarded growth rate.

All investigators showed evidence of the superiority of heat treated soybean meal to raw soybean meal when studies between the two forms of soybean meal were conducted. Either commercially solvent extracted soybean meal or laboratory autoclaved soybean meal has demonstrated the inactivation by heat treatment of the growth depression effect.

D. INFLUENCE OF AGE ON THE GROWTH DEPRESSION EFFECT OF RAW SOYBEAN MEAL:

The literature appears to be littered with contradictory reports of the influence of age in overcoming the depression effect of raw soybean meal. Bornstein et al. (1961) indicated that sensitivity of chicks to raw soybean meal decreased with an increase in age. Fisher et al. (1957) reported that the insensitivity to a growth depression effect in chickens is not evidenced until they are 14 weeks of age. Alumont and Nitsan (1961) reported that the intestinal proteolytic inhibition induced by raw soybeans lasts until the birds are three weeks of age and that at four weeks of age the proteolytic activity is recovering and reaching a normal level at six weeks of age.

Nesheim (1961) reported the disappearance of a marked inhibitory effect of raw soybeans on fat digestion at four weeks of age and indicated that the change was due to the effect of true age and was not an adaptation to raw soybean meal.

Saxena et al. (1963) reported that chicks of six weeks of age are not susceptible to the growth depression effect of raw soybean meal. On the contrary, Bornstein and Lipstein (1963) reported that age had no effect on the degree of susceptibility to the growth depression effect.

Saxena et al. (1963) also indicated that amino acid supplementation to counteract the growth depression effect in young chicks fed raw soybean meal diet was ineffective. Their findings agreed with those of Hill et al. (1953) and Booth et al. (1960) who reported

similar data, the former in chicks and the latter in rats, respectively.

However, the Saxena et al. (1963) data differed from those of Fisher and Johnson (1958), Borchers (1961), and Fisher and Shapiro (1963) who reported success in counteracting the growth depression effect of raw soybeans with amino acid supplementation.

Further, Saxena et al. (1963) differed with Bouthilet et al. (1950), and Brambila et al. (1961). The latter reported poor nitrogen retention in chicks fed raw soybean meal diet, whereas, Saxena et al. (1963) reported no effect on nitrogen retention.

E. OTHER DELETERIOUS EFFECT OF A RAW SOYBEAN MEAL DIET:

Miscellaneous deleterious effects have been reported from a raw soybean meal diet such as goiter in chicks (McCarrison, 1934; and Patton et al. 1939) and rats (Sharpless, 1938; and Sharpless et al. 1939). Such affected subjects developed hypertrophy of the thyroid glands in an attempt to increase iodine metabolism to make up for the iodine deficiency caused by goiter.

Cases of hypertrophy of the pancreas have been reported in the chick (Chernick et al. 1948; Bouthilet et al. 1950; Saxena et al. 1963; Lepkovsky et al. 1965)) and in the rat (Layman and Lepkovsky, 1957; Booth et al. 1960; and Haines and Layman, 1961)

Incidents of toxic substances in raw soybeans which, when injected into animals, cause fatality have also been reported (Falk 1914 in rabbits; Carnot and Gerard 1919 in dogs; Harris 1915 in guinea pigs; Liener and Pallansch 1952, and Liener 1953 in rats). Further, Liener

and Pallansch (1952) also reported a hemagglutinating factor. Liener (1953) called this toxin extracted from raw soybean meal "soyin".

IX. NUTRITIVE VALUE OF SUNFLOWER SEEDS AND SUNFLOWER MEAL

FOR POULTRY AND OTHER FARM ANIMALS

A. POULTRY:

The nutritive value of sunflower meal in chicks has been reported by a number of investigators (Grau and Almquist, 1945; Day and Levin, 1945; McGinnis et al., 1948; O'Neil, 1948; Morrison et al., 1953; Klain et al., 1956; Temperton et al., 1964; Thomas et al., 1965; Wessels, 1967; Waldroup et al., 1970; Dagher et al., 1980; and more recently by Alao and Balnave, 1984).

Data on egg laying performance of hens fed sunflower meal have also been reported (Tabakoff, 1939; Pettit et al., 1944; Hale and Brown, 1957; Walter et al., 1959; Rose, et al., 1972; and Uwayjan et al., 1983).

McGinnis et al., (1948) duplicated these data with chick growth trials. McGinnis et al., (1948) fed mixed sex New Hampshire chicks expeller processed soybean meal and sunflower meal in diets to supply a 21% crude protein level and reported the following results:

1. Both sunflower meal and soybean meal lacked an unidentified growth factor needed for maximum chick growth.
2. Soybean meal was found to be deficient in methionine.
3. Sunflower meal was found to be deficient in lysine.
4. Sunflower meal does not require supplementation with methionine.

The data reported by McGinnis et al., (1948) in general corroborated the findings of O'Neil (1948), Morrison et al., (1953), Klain

et al. (1956), Thomas et al. (1965), and Vaughan (1970) in that lysine is the first limiting amino acid in sunflower meal.

Mitchell et al. (1945) reported that sunflower seed protein had a lower biological value than properly heated soybean meal protein for rats, and Grau and Almquist (1945) showed that sunflower meal furnished all the essential amino acids required for chick growth when added at 20% of the total protein supplement in practical chick starter diets.

However, Cuca et al. (1973) reported a significant response from threonine supplementation in a broiler growth trial where a diet with 12% sunflower meal was supplemented with methionine, leucine, and threonine at graded levels. The data indicated that threonine could be a second limiting amino acid in sunflower protein.

Waldroup et al. (1970) indicated that 30% sunflower meal from high oil yielding varieties was satisfactory for broiler chick growth, provided such diets were pelleted. Further observations showed that in all mash diets the inclusion rate should not be more than 15% of the diet. Similar studies with sunflower meal were reported by Rad and Keshavarz (1976), Daghir et al. (1980), and Alao and Balnave (1984), respectively. Alao and Balnave (1984) compared the nutritive value of olive meal with sunflower meal and observed that sunflower meal is superior to olive meal. The broilers grew much faster with better feed efficiency and a higher concentration of body fat on sunflower protein than on olive protein.

Rad and Keshavarz (1976) indicated that 50% of the soybean meal protein could be replaced with sunflower meal protein without any

adverse effect on growth rate or feed efficiency. That amount was equivalent to 17.5% sunflower meal in the diet furnishing 7% total dietary protein.

Daghir et al. (1980) found no toxic effect from sunflower meal fed to broilers at a 20% level of the diet. They indicated that a dry heat treatment of the sunflower meal reduced broiler weight gains slightly more than steam heating.

Temperton et al. (1964a,b) investigated the phosphorus requirements of chicks fed all plant protein and indicated that replacement of soybean meal with sunflower meal on a protein equivalent basis at 6.5% of the chick starter or grower diet resulted in growth rate just as soybean alone and that the cost of feed was reduced. However, Klain et al. (1956) indicated that although sunflower meal is a rich source of niacin and choline, none-the-less, it is high in fibre, low in lysine, and moderately low in phenylalanine and tyrosine. Their studies showed that complete replacement of soybean meal with sunflower meal depressed growth rate in chicks.

The nutritive value of sunflower meal also has been investigated in a number of studies with egg laying hens (Hale and Brown, 1957; Walter et al. 1959; Rose et al. 1972; and Uwayjan et al. 1983).

Hale and Brown (1957) reported satisfactory egg production performance in all mash-grain layer diets with sunflower meal but remarked that the high fibre content in sunflower meal might cause problems in maintaining energy levels.

Walter et al. (1959) reported excellent egg production performance from all diets in which sunflower meal replaced 2 or 2.5% fish meal. Hen-day egg production, egg weight, and body weight maintenance were all satisfactory. The rations were fed at 9 and 13% crude protein levels.

Rose et al. (1972) reported a study in which layers were fed diets containing sunflower meal with graded levels of lysine supplementation as compared to the same levels in soybean protein and indicated the following results:

1. Hen-day egg production was reduced slightly when sunflower meal replaced soybean meal at the 50 or 100% level. The effect was more severe at the 100% level of replacement ($P < 0.01$).

2. Feed efficiency was reduced with sunflower meal ($P < 0.05$) being worst at the 100% replacement level of soybean meal.

3. The source of the sunflower meal influenced the results ($P < 0.01$). Hens on the sunflower meal source with the high fibre content ate more feed.

4. No difference was noted in mortality, but the hens on 100% sunflower meal protein were lighter than the rest.

5. No differences were noted in egg weight.

6. Egg stains were observed. This was shown to be due to chlorogenic acid which results in a characteristic blue-greenish stain. However, Rose et al. (1972) admitted that the problem of stains was due to faulty management. The eggs were stained with the feed and water mixture. Routine egg washing did not remove such stains.

7. Final body weights of the hens at the end of the experiment showed that hens on diets of 100% sunflower meal protein were lighter ($P < 0.05$). Also, final egg weights were reduced significantly for this same diet group ($P < 0.01$).

8. Egg production performance was reduced with 20-32% sunflower meal incorporation in the diet. Performance was poorest at the 27 and 32% levels.

9. Lysine contents of the sunflower meal diets were adequate or even higher than the N.R.C. requirement of 0.5% for laying birds. Lysine supplementation did not improve egg production performance consistently.

10. The problem of egg staining was overcome when the feed was fed in the crumbs form.

Uwayjan et al. (1983) evaluated the nutritive values of unprocessed whole sunflower seeds and soybean meal as to egg laying performance. All diets were yellow-corn based. Two experiments were conducted in floor pens with White Leghorn layers. Parameters measured were hen-day egg production, egg weight, feed consumption, feed efficiency, and body weight changes. In Experiment 1, 10, 20, and 30% of sunflower seeds replaced 15, 30, and 45% of soybean meal and 9.5, 19, and 28.6% of corn. Experiment 2 was conducted to evaluate the highest level of sunflower seeds that could replace soybean meal with 0.1 or 0.01 lysine plus 0.01% methionine supplementation. The following data were reported:

1. Hen-day egg production rates were slightly lower for the sunflower seed diets but statistically non-significant from the soybean controls. A drop in egg production was similar at the 10 or 20% levels but higher at the 30% level of sunflower meal.

2. Feed consumption dropped with the 10 and 30% levels of sunflowers ($P < 0.01$), but not with 20%.

3. Feed efficiency and egg weight were not affected by the sunflower seeds treatments, although there was a tendency for an increase in egg weight with an increase in sunflower seed levels.

4. Mortality was not affected.

The results of Experiment 2 did not show any beneficial response from either lysine or lysine plus methionine supplementation of 30% sunflower seed diets. Hens on the sunflower seed diets showed inferior performance compared to the control soybean meal group. These data agree with that of Rad *et al.* (1976) who failed to show responses from lysine when 50 or 100% of soybean meal was replaced with sunflower meal. The overall performance of the layers in the second was inferior as compared to the first experiment which was probably attributed to adverse winter weather conditions.

Studies to evaluate the nutritive value of sunflower meal in turkey growth trials have been reported by Slinger *et al.* (1949) and Gartley *et al.* (1950), respectively.

Slinger *et al.* (1949) compared the nutritive value of soybean and sunflower meals with Broad Breasted Bronze turkey poults. They indicated that sunflower meal as a sole protein source was seriously

deficient in the poult's lysine requirements. Further, they remarked that a mixture of equal parts of sunflower and soybean meals as the protein supplement was inadequate to support rapid early poult growth.

However, Gratley et al. (1950) reported contrary evidence in that sunflower meal, at a level of 21% or less in a 28% protein turkey poult starter diet, was satisfactory for the first four weeks and that 16.5% sunflower meal in a 26% protein diet supported adequate turkey growth from 4-8 weeks of age.

B. SWINE:

A number of investigations have been made to evaluate the nutritive value of either sunflower seeds or sunflower meal in swine nutrition (Cunha 1957, Delic et al. 1964, Laudert and Allee 1975, Dinusson et al. 1980a,b; Cook et al. 1980; Nielson and Aberne 1981; Kepler 1981; Thacker et al. 1984; and Hartman et al. 1985).

Delic et al. (1964) investigated the biological value of sunflower meal as a major source of protein for growing-finishing swine and indicated that although sunflower meal is a rich source of the amino acids tryptophan, arginine, and methionine, it is low in lysine. They concluded that it was inferior to soybean meal as the only source of protein supplement in swine rations.

Cunha (1957) reported that sunflower meal could furnish 20-30% of the total diet for growing swine and that levels above 30% depressed feed efficiency and growth rate.

Laudert and Allee (1975); Dinusson et al. (1980a,b); Cook et al. (1980); Kepler (1981); and Hartman et al. (1985) fed sunflower seeds to pigs.

Kepler (1981) fed gravid and lactating sows and gilts rations containing 25 and 50% sunflower seed and reported that three sows refused to eat the 50% sunflower seed diet. However, his overall data indicated no dietary effects on feed consumption.

Laudert and Allee (1975) fed sunflower seeds to growing-finishing swine in corn-soybean meal diets at 0, 20, 40, and 60% inclusion rates. The results showed a linear decrease in feed consumption and feed efficiency with increased sunflower seed. The fastest gains occurred with the control corn-soybean meal diet. Further observations showed an increase in the total unsaturated fatty acid content of back fat and longissimus muscle in swine fed the sunflower meal diets with the highest increase evident for linoleic acid. The linoleic acid concentration in back fat did not vary for the swine fed the various sunflower diets, but intramuscular fat increased with each increase in sunflower seed level.

Dinusson et al. (1980a) fed 0, 13, 26, and 39% sunflower seed levels in barley-soybean meal based diets and reported a decreased rate of gain at the 39% level. However, overall feed consumption and feed efficiency were not affected. In a second experiment Dinusson et al. (1980b) fed 0, 2.5, 5, and 10% sunflower seeds in barley-soybean based diets to evaluate diet palatability and rate of gain in growing-

finishing swine. They indicated no significant findings among the treatment groups.

Cook et al. (1980) reported on the dietary effect of sunflower seeds on pork carcass quality and indicated that sunflower seeds decreased carcass quality, carcass firmness, and marbling of the longissimus muscle.

Marchello et al. (1981) analyzed the fatty acid composition of the pork fat samples from Dinusson et al. (1980b). They assayed leaf fat, flank fat, the inner and outer layers of back fat, and intramuscular fat of the longissimus muscle. Analytical results indicated with increased sunflower seeds a relative decrease in myristic, palmitic, stearic, palmitoleic, oleic, and linolenic acids and an increase in linoleic, eicosadienoic, and arachidonic fatty acids. Linoleic acid showed the greatest proportional change with a mean range of from 13.6 to 48% of the total fatty acids present. Further observations indicated a positive correlation between linoleic acid content of sunflower seeds with back fat linoleic acid content.

Thacker et al. (1984) investigated amino acid availability and urea recycling in finishing swine fed barley-based diets supplemented with soybean or sunflower meal to supply 14% crude protein and reported the following results:

1. The availability of the essential amino acids as shown by ileal assay averaged 75.9% for the sunflower diet as against 74.7% for the soybean meal diet.

2. In this test availability of arginine, histidine, isoleucine, phenylalanine, glycine, and tyrosine were significantly higher for sunflower meal than soybean meal ($P < 0.05$).

3. The apparent availability of essential amino acids as shown by fecal assay averaged 80.2% for sunflower meal and 78.6% for soybean meal.

4. In this test methionine in sunflower meal had higher availability than that in soybean meal ($P < 0.05$).

5. There was no difference in nitrogen intake, fecal nitrogen, nitrogen digestibility, apparent protein utilization, and biological value of protein between the two protein sources. Observations with both diets showed approximately 32% of the nitrogen degraded in the hind gut.

6. Little or no difference in concentration of essential amino acids between the two protein sources with the exception of lysine. These findings agreed with earlier reports of Rossom *et al.* (1972); Shirley *et al.* (1973); Dinusson *et al.* (1980); and Nielsen and Abherne (1984).

7. The authors concluded that although significant differences in availability of essential amino acids between sunflower meal and soybean meal have been reported by Jorgensen *et al.* (1984), such differences could be corrected by the dilution effect of cereal grains added in the diet. Therefore, the lysine deficiency in sunflower meal not-with-standing; a 14% protein diet using sunflower meal supplemented

with synthetic lysine could furnish the amino acids required for optimum performance of finishing swine.

Jorgensen et al. (1984) also determined the availability of amino acids in soybean meal, fish meal, and meat and bone meal diets as major sources of protein when fed to growing pigs to supply 14% protein. Their results indicated that sunflower meal and soybean meal were at a par except for the lysine factor. With lysine disregarded, the order of amino acid availability was ranked in descending order as fish meal, soybean meal, sunflower meal, and meat and bone meal.

Hartman et al. (1985) conducted two experiments with growing-finishing swine to study the effect of sunflower seeds on growth performance, carcass quality, fatty acids composition, and consumer acceptability. In Experiment 1 sunflower seeds were fed at the 0, 5, 10, and 20% inclusion rate; and in Experiment 2 the levels were 0, 2.5, 5, and 10%. All the rations were corn-soybean meal based. The following data were reported:

1. Rate of gain increased with increased levels of sunflower seeds up to 5%, whereas variable results were observed at the 10% level. The combined data from the two experiments indicated slightly better performance as to rate of gain with the 10% level over the control group (0.77 kg ADG vs 0.745 kg ADG). Performance was worst at the 20% level.

2. Feed efficiency was not affected by the sunflower seeds addition. However, feed consumption was depressed significantly at the

10 and 20% levels in Experiment 1 but increased numerically in Experiment 2 with increasing levels of sunflower seeds.

3. Iodine numbers of fat indicated a decrease in carcass firmness with increasing levels of sunflower seed. Iodine numbers of fat from pigs fed 10 and 20% sunflower seeds were similar to the values reported by Wahlstrom et al. (1971) for a diet containing cooked, whole soybeans.

4. Fatty acid composition of back fat indicated a marked increase in linoleic acid and a decrease in other fatty acids as the dietary level of sunflower seeds was increased. This change also decreased the firmness of the carcass.

5. There were no dietary effects of sunflower seeds on other qualitative carcass characteristics, chemical composition, or taste panel scores. Neither was there any difference in the cutting quality of the pork among the dietary treatment groups.

6. The authors speculated that the decrease in carcass firmness of pigs fed high levels of sunflower seeds could make it less acceptable for normal processing procedures but concluded that it might not affect meat packer processing procedures.

C. RUMINANTS:

Few studies have been reported on the nutritive value of sunflower meal for ruminants (Pearson et al. 1954; Sarbason 1965; Harvey 1970; and Amos et al. 1974).

Pearson et al. (1954) compared the nutritive value of cottonseed meal protein with sunflower meal protein in fattening steer

formaldehyde treated soybean meal ($P < 0.05$) while those of the sunflower meal, though not significant, were higher as compared to soybean meal.

4. Data obtained from enzymatic and rumen fluid in vitro studies indicated that ammonia release was higher for sunflower meal than for soybean meal. The probable explanation for that was attributed to the presence of chlorogenic, caffeic, and guinic acids in sunflower meal which agreed with earlier reports of Pomenta and Burns (1971) and Milic et al. (1968). Milic et al. (1968) reported that the chlorogenic acid in sunflower meal decreased trypsin digestion activity of sunflower meal protein. Pomenta and Burns (1968) indicated that these acids react with protein in the same manner as the tannic acids by preventing microbial degradation of the sunflower meal protein in the rumen.

X. NUTRITIVE VALUE OF MEAT MEAL AND MEAT AND BONE MEAL

IN POULTRY AND OTHER FARM ANIMALS

A. POULTRY:

The nutritive values of meat scraps for chick growth were reported by Kraybill and Wilder (1947); March et al. (1949); March et al. (1950); Patrick (1953); and Summar et al. (1964). Summar et al. (1964) reported deficiencies of six amino acids with the limitations in order being methionine, tryptophan, isoleucine, cystine, threonine, and arginine, respectively. Their investigation was with White Leghorn male chicks with meat scraps or soybean meal as the major protein source. The diet with meat scrap protein failed to support a satisfactory growth rate. They attributed the probable cause of the poor performance of meat scrap meal to excess calcium and phosphorus in the diet.

A few studies on meat and bone meal also have been reported by Kratzer and Davis (1959) and Johri et al. (1980) as evaluated by chick growth rate assays.

Kratzer and Davis (1959) fed Single Comb White Leghorn chicks ten different sources of meat and bone meal and found the following results:

1. Chicks on the number one source responded markedly only to the addition of methionine and little to either tryptophan or isoleucine supplementation. The addition of all three limiting amino acids caused the chicks to respond with greater improvement in growth rate than from methionine alone. Supplementing the diet with higher

levels of the three amino acids failed to elicit any further response in growth rate. From the data it was concluded that the sample of meat and bone meal to which blood was added was obviously deficient in sulfur amino acids.

2. Chicks on the number two source showed improvement with methionine and tryptophan additions but did not respond to isoleucine, leucine, valine, arginine, or lysine additions. The conclusion was that the meal was adequate in those amino acids.

3. Chicks on the number three source gave responses similar to those on number two, responding only to methionine and tryptophan additions.

4. The number four source supported reasonable growth without addition of the limiting amino acids.

5. The number five source gave poor growth without the limiting amino acid additions.

6. The number six source supported reasonable chick growth without the limiting amino acid additions, but slight improvements were observed with supplementation of either cystine or methionine.

7. Meat and bone meal samples 7, 8, 9, and 10 were all found to be deficient in the sulfur amino acids and tryptophan.

The Kratzer and Davis (1959) data agreed with the earlier reports by Kraybill and Wilder (1947) in that meat and bone meal could be improved with additions of methionine and tryptophan. Also, Kratzer and Davis (1959) were in further agreement with Grau and Almquist (1944) in that blood meal is deficient in isoleucine for chicks.

However, Kratzer and Davis (1959) did not show deficiencies of lysine, leucine, valine, or arginine in meat protein. This is contrary to reports from March et al. (1950) and Patrick (1953) that showed lysine was the first limiting amino acid in practical diets containing meat meal.

Johri et al. (1980) investigated the nutritive value of four meat and bone meal samples after analysis for methionine, tryptophan, lysine, and orange-G dye binding power. They were fed as the major source of protein supplement in starch or cereal based diets for broilers. The results indicated difficulty in correlating chick growth with the analytical chemical indices. Generally, the relative growth rates were similar irrespective of cereal addition. A second study involved nine meat and bone meal samples with milo, wheat, or corn based diets. They found that chick growth was relatively similar irrespective of the type of cereal grain used.

In conclusion, they demonstrated from their data that biological testing might have better valid application in evaluating the nutritive value of meat and bone meal in cereal based diets than the use of chemical analytical indices.

B. SWINE:

Several studies have been reported on the nutritive value of meat scraps and meat and bone meal in swine nutrition (Bloss et al. 1953; Terrill et al. 1954; Meade and Teter 1957; Becker and Jensen 1961; Peo and Hudman 1962; and Luce et al. 1964).

Luce et al. (1964) conducted three experiments with a total of 234 crossbred pigs to evaluate the nutritive value of meat and bone meal in comparison to soybean meal. All diets were corn based. Meat and bone meal was fed at the 5 and 10% rate of inclusion. A second objective was to determine the effects of supplementation with lysine, methionine, and tryptophan. The data collected from the three experiments indicated that supplementation of amino acids failed to support any significant rate of gain in growth rate. But tryptophan, in most instances, did give improved rate of gain.

Average daily feed consumption decreased in Experiment 1 and 3 when lysine was supplemented in the 10% level meat and bone meal diet.

The authors concluded that meat and bone meal with or without amino acid supplementation was inferior to soybean meal protein but remarked that meat and bone meal could effectively replace part of soybean meal protein. Similar poor growth performance data in growing-finishing trials with meat and bone meal were earlier reported by Bloss et al. (1953), Meade and Teter (1957), Becker and Jensen (1961), and Peo and Hudman (1962).

EXPERIMENTAL DESIGN AND PROCEDURE

In each of two experiments a "randomized complete block design" was used. Experiment 1 had a total of 864 Babcock--300 pullets randomly distributed into cages for twelve treatments. Each treatment was replicated six-times, and each replicate consisted of twelve birds. The birds were in twelve-inch single cages, with four birds per cage. The twelve diets were formulated with soybean meal, sunflower meal, or meat and bone meal as the major sources of protein in yellowcorn-based diets. The diets provided four levels of protein; 16%, 14%, 12%, or 12% supplemented with essential amino acids. All the diets except the 12% protein without amino acids supplementation were formulated to furnish at least the minimum N. R. C. (1977) requirements for the sulfur amino acids and lysine. Energy levels were maintained with yellow grease for the 16% and 14% protein sunflower meal diets. Water and feed were provided ad libitum, and seventeen hours of light were provided per day.

Parameters measured were hen-day egg production, daily feed consumption, feed efficiency, egg weight, egg shell-thickness, Haugh units, mortality, and final body weight. A period consisted of 28 days. Data on the above parameters were collected daily except egg shell-thickness, Haugh units and egg weight which were recorded at the end of each period and the final body weight at the end of the experiment.

Experiment 1 lasted for 10 periods; it started on January 14, 1981, and terminated on October 22, 1981. At the end of the experiment

the birds were weighed and pooled blood samples were taken for amino acids analyses, and also pooled egg albumen samples were taken for amino acids analyses.

Experiment 2 was conducted to investigate the possibility of utilizing meat and bone meal in reasonably greater proportions, as a major source of protein in practical layer diets beyond its traditional role in smaller amounts as a source of calcium and phosphorus supplementation. A second concern was to re-evaluate the comparative nutritive value of soybean meal and sunflower meal in supporting maximum egg production performance. In this case a sunflower meal of greater crude protein content with lesser fibre relative to the quality of the sunflower meal used in the first experiment was used.

Experiment 2 involved a total of 720 Shaver--288 pullet layers, randomly distributed into cages. Each of ten treatments was replicated six-times, and each replicate had twelve birds housed in cages similar to in Experiment 1. All environmental conditions furnished and the parameters measured were exactly the same as in Experiment 1. This Experiment 2 involved mixed diets with either soybean meal plus meat and bone meal or sunflower meal plus meat and bone meal as the major sources of protein in yellow corn-based diets. Further, the diets were formulated to provide only 16% or 12% protein, and the 12% protein diet was supplemented with the essential amino acids methionine and lysine. All diets met the minimum N.R.C. (1977) requirements. Finally, Experiment 2 lasted for thirteen periods, being started on February 26,

1982, and ended on February 24, 1983. Methods for collection of data were exactly the same as in Experiment 1.

The 12% protein diets supplemented with amino acids formed the control group in each experiment. The basal diets for Experiments 1 and 2 with calculated analyses are shown in Tables 1 through 6.

TABLE 1. COMPOSITION OF DIETS
(EXPERIMENT 1)

INGREDIENTS	SOYBEAN MEAL PROTEIN LEVEL				SUNFLOWER MEAL PROTEIN LEVEL			
	16	14	12 WITHOUT AA	12 + AA (CONTROL)	16	14	12 WITHOUT AA	12 + AA (CONTROL)
YELLOW CORN (8.9% CP)	67.3	72.4	77.6	77.6	50.3	60.0	72.0	72.0
SOYBEAN MEAL (48% CP)	20.2	15.1	9.9	9.9	----	----	----	----
SUNFLOWER MEAL (29.1% CP)	----	----	----	----	32.0	24.5	15.5	15.1
MEAT AND BONE MEAL (50% CP)	----	----	----	----	----	----	----	----
DEHYDRATED ALFALFA (17% CP)	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
DICALCIUM PHOSPHATE (24% Ca) & (18% P)	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
LIMESTONE (33% Ca)	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0
YELLOW GREASE	----	----	----	----	4.3	3.0	----	----
VITAMIN PREMIX ¹	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
SALT ²	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
DL-METHIONINE	----	----	----	0.05	----	----	----	0.04
L-LYSINE	----	----	----	0.12	----	0.1	----	0.2

¹ Supplied per kg. of diet: vitamin A, 5280 U.S.P.; vitamin D₃, 1375 U.S.P.; vitamin E, 22 I.U.; vitamin B₁₂, 0.0088 mg.; niacin, 44 mg.; choline chloride, 440 mg.; riboflavin, 6.6 mg.; d-calcium pantothenic acid, 8.8 mg.; vitamin K, 1.1 mg.; folic acid, 1.1 mg.; and biotin, 0.11 mg.

² Supplied per kg. of diet: sodium chloride, 4.8g.; zinc, 18 mg.; iron, 10 mg.; manganese, 10 mg.; magnesium, 7.5 mg.; copper, 1.5 mg.; cobalt, 0.25 mg.; and iodine, 0.35 mg.

TABLE 1. COMPOSITION OF DIETS, (cont.)
(EXPERIMENT 1)

INGREDIENTS	MEAT AND BONE MEAL PROTEIN LEVEL			
	16	14	12 WITHOUT AA	12 + AA (CONTROL)
YELLOW CORN (8.9% CP)	75.0	78.7	82.1	82.1
SOYBEAN MEAL (48% CP)	---	---	---	---
SUNFLOWER MEAL (29.1% CP)	---	---	---	---
MEAT AND BONE MEAL (50% CP)	18.0	13.3	8.7	8.7
DEHYDRATED ALFALFA (17% CP)	2.0	2.0	2.0	2.0
DICALCIUM PHOSPHATE (24% Ca) & (18% P)	---	---	---	---
LIMESTONE (33% Ca)	4.0	5.0	6.2	6.2
YELLOW GREASE	---	---	---	---
VITAMIN PREMIX ¹	0.5	0.5	0.5	0.5
SALT ²	0.5	0.5	0.5	0.5
DL-METHIONINE	---	0.08	---	0.08
L-LYSINE	---	0.1	---	0.2

¹ Supplied per kg. of diet: vitamin A, 5280 U.S.P.; vitamin D₃, 1375 U.S.P.; vitamin E, 22 I.U.; vitamin B₁₂, 0.0088 mg.; niacin, 44 mg.; choline chloride, 440 mg.; riboflavin, 6.6 mg.; D-calcium pantothenic acid, 8.8 mg.; vitamin K, 1.1 mg.; folic acid, 1.1 mg.; and biotin, 0.11 mg.

² Supplied per kg. of diet: sodium chloride, 4.8 g.; zinc, 18 mg.; iron, 10 mg.; manganese, 10 mg.; magnesium, 7.5 mg.; copper, 1.5 mg.; cobalt, 0.25 mg.; and iodine, 0.35 mg.

TABLE 2. CALCULATED ANALYSIS OF DIETS

(EXPERIMENT 1)

INGREDIENTS	SOYBEAN MEAL PROTEIN LEVEL				SUNFLOWER PROTEIN LEVEL			
	16	14	12 WITHOUT AA	12 + AA (CONTROL)	16	14	12 WITHOUT AA	12 + AA (CONTROL)
ENERGY—ME/KG	2811	2854	2914	2914	2567	2672	2777	2777
METHIONINE AND CYSTINE, %	0.56	0.51	0.45	0.5	0.6	0.53	0.46	0.5
LYSINE, %	0.8	0.6	0.5	0.6	0.7	0.6	0.4	0.6
TRYPTOPHAN, %	0.2	0.17	0.15	0.15	0.25	0.21	0.17	0.17
ISOLEUCINE, %	0.8	0.7	0.6	0.6	1.03	0.9	0.7	0.7
CALCIUM, %	3.34	3.32	3.28	3.28	3.4	3.37	3.34	3.34
PHOSPHORUS, %	0.57	0.56	0.56	0.56	0.64	0.62	0.59	0.59
FIBRE, %	2.44	2.38	2.30	2.30	10.03	8.05	6.07	6.07

TABLE 2. CALCULATED ANALYSIS OF DIETS (cont)

(EXPERIMENT 1)

	MEAT AND BONE MEAL PROTEIN LEVEL			
	16	14	12 WITHOUT AA	12 + AA (CONTROL)
ENERGY--ME/KG	2915.5	2946.7	2970.5	2970.5
METHIONINE AND CYSTINE, %	0.51	0.5	0.42	0.5
LYSINE, %	0.6	0.6	0.4	0.6
TRYPTOPHAN, %	0.13	0.12	0.11	0.11
ISOLEUCINE, %	0.6	0.6	0.5	0.5
CALCIUM, %	3.47	3.36	3.32	3.32
PHOSPHORUS, %	0.99	0.76	0.53	0.53
FIBRE, %	2.34	2.32	2.29	2.29

TABLE 3. COMPOSITION OF DIETS
(EXPERIMENT 2)

INGREDIENTS	SOYBEAN MEAL PROTEIN LEVEL		SUNFLOWER MEAL PROTEIN LEVEL		MEAT AND BONE MEAL PROTEIN LEVEL	
	16	12 (CONTROL)	16	12 (CONTROL)	16	12 (CONTROL)
YELLOW CORN (8.9% CP)	66.0	75.77	53.97	70.52	75.0	81.0
SOYBEAN MEAL (48% CP)	20.5	10.5	----	----	----	----
SUNFLOWER MEAL (32% CP)	----	----	32.0	16.0	----	----
MEAT AND BONE MEAL (50% CP)	----	----	----	----	18.0	9.0
DEHYDRATED ALFALFA (17% CP)	2.0	2.0	2.0	2.0	2.0	2.0
DICALCIUM PHOSPHATE (24% Ca) & (18% P)	2.5	2.5	2.5	2.5	----	----
LIMESTONE (33% Ca)	8.0	8.0	7.0	8.0	4.0	7.0
YELLOW GREASE	----	----	1.5	----	----	----
VITAMINE PREMIX ¹	0.5	0.5	0.5	0.5	0.5	0.5
SALT ²	0.5	0.5	0.5	0.5	0.5	0.5
DL-METHIONINE	----	0.13	----	0.06	----	0.08
L-LYSINE	----	0.10	0.03	0.22	----	0.20

¹ Supplied per kg. of diet: vitamin A, 5280 U.S.P.; vitamin D₃, 1375 U.S.P.; vitamin E, 22 I.U.; vitamin B₁₂, 0.0088 mg.; niacin, 44 mg.; choline chloride, 440 mg.; riboflavin, 6.6 mg.; d-calcium pantothenic acid, 8.8 mg.; vitamin K, 1.1 mg.; folic acid, 1.1 mg.; and biotin, 0.11 mg.

² Supplied per kg. of diet: sodium chloride, 4.8 g.; zinc, 18 mg.; iron, 10 mg.; manganese, 10 mg.; magnesium, 7.5 mg.; copper, 1.5 mg.; cobalt, 0.25 mg.; and iodine, 0.35 mg.

TABLE 4. CALCULATED ANALYSIS OF DIETS
(EXPERIMENT 2)

INGREDIENTS	SOYBEAN MEAL PROTEIN LEVEL •		SUNFLOWER MEAL PROTEIN LEVEL		MEAT AND BONE MEAL PROTEIN LEVEL	
	16	12 (CONTROL)	16	12 (CONTROL)	16	12 (CONTROL)
ENERGY—ME/KG	2774	2858	2713	2777	2916	2940
METHIONINE AND CYSTINE, %	0.56	0.50	0.54	0.50	0.51	0.50
LYSINE, %	0.8	0.63	0.60	0.60	0.60	0.60
TRYPTOPHAN, %	0.2	0.15	0.22	0.16	0.13	0.11
ISOLEUCINE, %	0.8	0.6	0.9	0.6	0.6	0.5
CALCIUM, %	3.3	3.3	3.3	3.3	3.3	3.3
PHOSPHORUS, %	0.57	0.56	0.53	0.58	1.00	0.55
FIBRE, %	2.42	2.32	8.29	5.25	2.52	2.37

TABLE 5. COMPOSITION OF MIXED PROTEIN DIETS
(EXPERIMENT 2)

INGREDIENTS	SOYBEAN MEAL AND MEAT AND BONE MEAL PROTEIN LEVEL		SUNFLOWER MEAL AND MEAT AND BONE MEAL PROTEIN LEVEL	
	16	12 (CONTROL)	16	12 (CONTROL)
YELLOW CORN (8.9% CP)	73.0	78.73	69.0	74.24
SOYBEAN MEAL (48% CP)	4.0	4.5	----	----
SUNFLOWER MEAL (32% CP)	----	----	8.0	9.0
MEAT AND BONE MEAL (50% CP)	15.0	5.0	15.0	5.0
DEHYDRATED ALFALFA (17% CP)	2.0	2.0	2.0	2.0
DICALCIUM PHOSPHATE (24% Ca) & (18% P)	----	1.5	----	15.
LIMESTONE (33% Ca)	5.0	7.0	5.0	7.01
VITAMIN PREMIX ¹	0.5	0.5	0.5	0.5
SALT ²	0.5	0.5	0.5	0.5
DL-METHIONINE	----	0.07	----	0.06
L-LYSINE	----	0.2	----	0.2

¹ Supplied per kg. of diet: vitamin A, 5280 U.S.P.; vitamin D₃, 1375 U.S.P.; vitamin E, 22 I.U.; vitamin B₁₂, 0.0088 mg.; niacin, 44 mg.; choline chloride, 440 mg.; riboflavin, 6.6 mg.; D-calcium pantothenic acid, 8.8 mg.; vitamin K, 1.1 mg.; folic acid, 1.1 mg.; and biotin, 0.11 mg.

² Supplied per kg. of diet: sodium chloride, 4.8 g.; zinc, 18 mg.; iron, 10 mg.; manganese, 10 mg.; magnesium, 7.5 mg.; copper, 1.5 mg.; cobalt, 0.25 mg.; and iodine, 0.35 mg.

TABLE 6. CALCULATED ANALYSIS OF MIXED PROTEIN DIETS

INGREDIENTS	(EXPERIMENT 2)			
	SOYBEAN MEAL AND		SUNFLOWER MEAL AND	
	FEAT AND BONE MEAL		FEAT AND BONE MEAL	
	PROTEIN LEVEL		PROTEIN LEVEL	
	16	12 (CONTROL)	16	12 (CONTROL)
ENERGY ME/KG	2890	2907	2797	2800
METHIONINE AND CYSTINE, %	0.52	0.50	0.53	0.50
LYSINE, %	0.70	0.60	0.60	0.60
TRYPTOPHAN, %	0.15	0.13	0.16	0.12
ISOLEUCINE, %	0.7	0.6	0.6	0.6
CALCIUM, %	3.3	3.3	3.3	3.2
PHOSPHORUS, %	0.85	0.60	0.86	0.63
FIBRE, %	2.36	2.30	3.84	3.98

METHOD OF STATISTICAL ANALYSIS

OF DATA COLLECTED

All the data collected in both experiments were subjected to the statistical analyses of variance method according to the procedures described by Steel and Torrie (1980), and the treatment means were separated for significance according to Duncan's multiple range test (1955).

BLOOD SERUM SAMPLE PREPARATION AND

CHEMICAL ANALYTICAL PROCEDURE FOR AMINO ACID CONTENT

Towards the end of each experiment, pooled whole blood samples were taken randomly from each replicate group by cardiac puncture, into centrifuge tubes containing sodium citrate. The function of the sodium citrate was to prevent coagulation of the blood. The whole blood samples were taken in the afternoon. The blood samples were immediately centrifuged at 10,000 r.p.m. for ten minutes. The resultant serum from each treatment group was pooled into three replicates and stored in a cold room at 0°C.

A 0.3M lithium citrate buffer (pH 2.8) was prepared with 3.75% sulfo-salicylic acid (SSA) and adjusted to pH 1.8 with lithium hydroxide. One vol. of the serum was mixed with 4 vol. of the buffer and was centrifuged in a refrigerated centrifuge at 10,000 r.p.m. for 10 minutes at 0°C. One ml. of the supernatant fluid was subjected to amino acid analysis in a Beckman 120 C automatic amino acid analyzer. The concentrations of amino acid content were reported as micromoles per ml.

EGG ALBUMEN SAMPLE PREPARATION AND
CHEMICAL ANALYTICAL PROCEDURE
FOR AMINO ACID ASSAY

Towards the end of each experiment five eggs were randomly taken from each replicate group. These were broken out, and the egg whites were carefully sieved from the egg yokes. A single pooled sample was made for each treatment group, sealed in plastic bags, and kept frozen in a deep freezer.

One gram of each pooled sample was put into a 100 ml flask and hydrolysed with 100 ml. of 6N hydrochloric acid for 24 hours at 110° C under an atmosphere of nitrogen gas. The samples were evaporated to dryness and washed three times with deionized water in order to remove all hydrochloric acid present. The dried samples were dissolved in .1 ml of pH 2.2 (0.02 N sodium citrate) diluting buffer. The samples were filtered and 1 ml. of each sample was subjected to the automatic (Beckman 120 C) amino acid analyzer for analyses of amino acids content. Amino acids concentrations were reported as micromoles per gram.

RESULTS

EXPERIMENT (1)

HEN-DAY EGG PRODUCTION:

The statistical analysis for the ten 28-day periods showed that hens on the diet with soybean protein supplement laid significantly more eggs than hens fed sunflower meal or meat and bone meal ($P < 0.05$) at both the 16% and 14% protein levels. Also, hens fed sunflower meal out-performed those fed meat and bone meal ($P < 0.05$). There was no significant difference between hens fed soybean meal or hens fed sunflower meal to provide the 12% protein plus amino acid diets. There was significant difference between the performance with the 16% and 14% soybean meal diets ($P < 0.05$). Also, hens fed sunflower meal to provide the 14% protein level under this experiment out-performed those fed the 16% protein level. The 14% protein diets had a higher energy content, and performance of hens fed the 12% control diet with amino acid supplementation was next to that of the 14% protein-fed hens. There was no significant difference between hens fed the 14% and the 12% protein plus amino acid supplemented sunflower meal diets. Hens fed meat and bone meal showed poor performance at all protein levels, with performance decreasing with each decrease in protein level. There was no difference in performance of hens fed the 12% protein diets with or without amino acid supplementation.

In the overall consideration of main effects, there was no difference between hens fed the 16% or 14% protein diet as to hen-day egg production performance. But a significant difference did exist between

hens fed the 16 or 14% protein diets and those on the 12% level with or without amino acid supplementation ($P < 0.05$). Hen-day egg production data are presented in Table 7.

TABLE 7
HEN-DAY EGG PRODUCTION

Protein level	12% protein	14% protein	16% protein
Control	10.2	10.5	10.8
Supplemented	10.5	10.8	11.0
12% protein	10.2	10.5	10.8
14% protein	10.5	10.8	11.0
16% protein	10.8	11.0	11.2

TABLE 7. HEN-DAY EGG PRODUCTION AS AFFECTED BY PROTEIN SOURCE AND LEVEL
(EXPERIMENT 1)

PROTEIN SOURCE	PROTEIN LEVEL				PROTEIN SOURCE MEAN
	16	14	12 WITHOUT AA	12 + AA (CONTROL)	
SOYBEAN MEAL	63.3 ^c	69.8 ^c	64.2 ^c	65.9 ^b	65.8 ^F
SUNFLOWER MEAL	61.6 ^b	66.9 ^b	51.8 ^b	65.4 ^b	61.43 ^E
MEAT AND BONE MEAL	58.2 ^a	48.0 ^a	38.0 ^a	39.2 ^a	45.85 ^D
PROTEIN LEVEL MEAN	63.0 ^C	62.0 ^C	51.0 ^A	57.0 ^B	----

a,b,c WITHIN COLUMNS, MEANS WITH UNLIKE SUPERSCRIPTS ARE SIGNIFICANTLY DIFFERENT (P<0.05)

A,B,C MEANS WITH UNLIKE SUPERSCRIPTS ARE SIGNIFICANTLY DIFFERENT (P<0.05)

D,E,F MEANS WITH UNLIKE SUPERSCRIPTS ARE SIGNIFICANTLY DIFFERENT (P<0.05)

DAILY FEED CONSUMPTION AND FEED EFFICIENCY

Hens fed the sunflower meal diets consumed significantly more feed than the other groups ($P < 0.05$). Feed consumption was significantly depressed with hens fed the meat and bone meal treatments ($P < 0.05$). Feed conversion also was significantly poorer for hens fed meat and bone meal diets as compared to those fed soybean or sunflower meal. Hens on the soybean meal diets showed increased feed intakes and feed efficiencies. However, both feed intake and feed efficiency were significantly poorer for hens fed 12% protein as compared to hens fed either the 16 or 14% protein levels. Further, hens on the 12% amino acid supplemented diets showed significantly better performance over hens on the 12% diets without amino acid supplementation ($P < 0.05$) as to feed consumption and feed efficiency.

There was a significant interaction between protein level and feed consumption with regards to hens fed the meat and bone meal diets ($P < 0.01$) with a progressive decline in feed intake as the protein level was decreased. The effect was most pronounced at the 12% protein level with or without amino acid supplementation; feed efficiency also followed the same pattern. Feed consumption and feed efficiency data are shown in Tables 8 and 9 respectively.

TABLE 8. DAILY FEED CONSUMPTION AS AFFECTED BY PROTEIN SOURCE AND LEVEL
(EXPERIMENT 1)

PROTEIN SOURCE	PROTEIN LEVEL				PROTEIN SOURCE MEAN
	16	14	12 WITHOUT AA	12 + AA (CONTROL)	
	Gm.	Gm.	Gm.	Gm.	Gm.
SOYBEAN MEAL	107 ^b	105 ^b	105 ^b	103 ^b	105 ^E
SUNFLOWER MEAL	110 ^c	110 ^c	103 ^b	111 ^c	109 ^F
MEAT AND BONE MEAL	98 ^a	89 ^a	88 ^a	83 ^a	90 ^D
PROTEIN LEVEL MEAN	105 ^B	101 ^B	97 ^A	99 ^A	—

a,b,c WITHIN COLUMNS, MEANS WITH UNLIKE SUPERSCRIPTS ARE SIGNIFICANTLY DIFFERENT (P<0.05)

A,B MEANS WITH UNLIKE SUPERSCRIPTS ARE SIGNIFICANTLY DIFFERENT (P<0.05)

D,E,F MEANS WITH UNLIKE SUPERSCRIPTS ARE SIGNIFICANTLY DIFFERENT (P<0.05)

TABLE 9. FEED EFFICIENCY AS AFFECTED BY PROTEIN SOURCE
AND LEVEL
(EXPERIMENT 1)

PROTEIN SOURCE	PROTEIN LEVEL				PROTEIN SOURCE MEAN
	16	14	12 WITHOUT AA	12 + AA (CONTROL)	
(GMS EGG PER 100 GMS. FEED)					
SOYBEAN MEAL	40.2 ^b	41.8 ^c	38.5 ^c	38.0 ^b	40.0 ^F
SUNFLOWER MEAL	39.5 ^b	38.5 ^b	30.0 ^b	36.2 ^b	36.0 ^E
MEAT AND BONE MEAL	35.5 ^a	32.0 ^a	24.0 ^a	26.0 ^a	24.0 ^D
PROTEIN LEVEL MEAN	38.3 ^C	37.4 ^C	30.8 ^A	34.0 ^B	---

a,b,c WITHIN COLUMNS, MEANS WITH UNLIKE SUPERSCRIPTS ARE SIGNIFICANTLY DIFFERENT (P<0.05)

A,B,C MEANS WITH UNLIKE SUPERSCRIPTS ARE SIGNIFICANTLY DIFFERENT (P<0.05)

D,E,F MEANS WITH UNLIKE SUPERSCRIPTS ARE SIGNIFICANTLY DIFFERENT (P<0.05)

EGG WEIGHT:

Egg weight is measured in grams, and it is influenced by a number of factors of which dietary source of protein and levels of amino acids and linoleic acid are considered important.

The egg sizes laid by hens fed the meat and bone meal diets were significantly smaller compared to either of those from hens fed sunflower or soybean meal respectively ($P < 0.05$). The egg sizes of the sunflower meal fed hens and those of soybean meal were similar. There was no significant dietary protein level influence on egg weight, except that hens fed the 12% protein diets without amino acid supplementation laid smaller eggs. Egg weight data are shown in Table 10.

HAUGH UNITS AND EGG SHELL THICKNESS:

The Haugh unit is a measurement for egg albumen quality. There was no significant difference between the Haugh units of eggs laid by hens on the meat and bone meal diets compared to those on sunflower meal diets; however, the values for eggs from hens on meat and bone meal diets showed a slight numerical superiority. However, the Haugh unit values of eggs laid by hens on meat and bone diets were significantly better than those of soybean meal diets ($P < 0.05$). There was no significant difference between Haugh units of eggs laid by hens fed soybean or sunflower meal diets, though the latter diets showed a slightly higher numerical value than the former. There was no influence of dietary protein levels on Haugh units.

TABLE 10. BGG WEIGHT AS AFFECTED BY PROTEIN SOURCE AND LEVEL
(EXPERIMENT 1)

PROTEIN SOURCE	PROTEIN LEVEL				PROTEIN SOURCE MEAN
	16	14	12 WITHOUT AA	12 + AA (CONTROL)	
	gm.	gm.	gm.	gm.	gm.
SOYBEAN MEAL	65.8 ^a	65.0 ^a	64.4 ^a	64.8 ^a	65.0 ^D
SUNFLOWER MEAL	66.0 ^a	65.5 ^a	64.2 ^a	64.2 ^a	64.6 ^D
MEAT AND BONE MEAL	62.8 ^b	62.9 ^b	60.5 ^b	60.2 ^b	61.6 ^E
PROTEIN LEVEL MEAN	64.9 ^A	64.5 ^A	62.5 ^B	63.1 ^{B,A}	---

a,b WITHIN COLUMNS, MEANS WITH UNLIKE SUPERSCRIPITS ARE SIGNIFICANTLY DIFFERENT (P<0.05)

A,B MEANS WITH UNLIKE SUPERSCRIPITS ARE SIGNIFICANTLY DIFFERENT (P<0.05)

D,E MEANS WITH UNLIKE SUPERSCRIPITS ARE SIGNIFICANTLY DIFFERENT (P<0.05)

Egg shell-thickness is measured in millimeters and reported as $\text{mm} \times 100$. It is an important economic factor for processing and marketing of eggs. There was practically little or no difference in egg shell-thickness of eggs laid by hens on all dietary protein supplements, and neither did dietary protein level had any effect. Haugh units and egg shell thickness data are presented in Tables 11 and 12 respectively.

TABLE 11. HAUGH UNITS AS AFFECTED BY PROTEIN SOURCE AND LEVEL

(EXPERIMENT 1)

PROTEIN SOURCE	PROTEIN LEVEL				PROTEIN SOURCE MEAN
	16	14	12 WITHOUT AA	12 + AA (CONTROL)	
SOYBEAN MEAL	67.0 ^a	69.0 ^a	72.0 ^b	68.0 ^a	69.0 ^D
SUNFLOWER MEAL	71.0 ^b	73.0 ^b	68.0 ^a	71.0 ^b	70.8 ^{D,E}
MEAT AND BONE MEAL	72.0 ^b	67.0 ^a	73.0 ^b	75.0 ^c	71.8 ^E
PROTEIN LEVEL MEAN	70.0 ^A	70.0 ^A	71.0 ^A	71.0 ^A	---

a,b,c WITHIN COLUMNS, MEANS WITH UNLIKE SUPERSSCRIPTS ARE SIGNIFICANTLY DIFFERENT (P<0.05)

A MEANS WITH UNLIKE SUPERSSCRIPTS ARE SIGNIFICANTLY DIFFERENT (P<0.05)

D,E MEANS WITH UNLIKE SUPERSSCRIPTS ARE SIGNIFICANTLY DIFFERENT (P<0.05)

TABLE 12. EGG SHELL THICKNESS
AS AFFECTED BY PROTEIN SOURCE AND LEVEL
(EXPERIMENT 1)

PROTEIN SOURCE	PROTEIN LEVEL,				PROTEIN SOURCE MEAN
	16	14	12 WITHOUT AA	12 + AA (CONTROL)	
	mm x 100	mm x 100	mm x 100	mm x 100	mm x 100
SOYBEAN MEAL	37.34 ^a	35.93 ^a	36.57 ^a	35.86 ^a	37.0 ^D
SUNFLOWER MEAL	36.30 ^a	37.49 ^a	36.61 ^a	37.3 ^a	36.3 ^D
MEAT AND BONE	37.73 ^a	37.7 ^a	37.41 ^a	36.4 ^a	37.1 ^D
PROTEIN LEVEL MEAN	37.1 ^A	37.0 ^A	36.9 ^A	36.5 ^A	---

WITHIN COLUMNS, MEANS WITH SAME SUPERSCRIPTS ARE NONSIGNIFICANTLY DIFFERENT ($P < 0.05$)

MEANS WITH SAME SUPERSCRIPTS ARE NONSIGNIFICANTLY DIFFERENT ($P < 0.05$)

MEANS WITH SAME SUPERSCRIPTS ARE NONSIGNIFICANTLY DIFFERENT ($P < 0.05$)

MORTALITY:

Mortality is expressed as percent of dead birds over live birds at the end of each 28-day period. There was no significant difference among hens fed the soybean, sunflower, or meat and bone meal supplement diets in mortality. There was no interaction between protein level and percent mortality. However, in this experiment the cumulative main effect due to protein level indicated that hens fed the 14% protein diet and the 12% protein with amino acid supplementation diet showed the lowest mortality compared to those fed either the 16% protein diet or the 12% protein diet without amino acid supplementation ($P < 0.05$). There was no mortality difference between hens fed the 16% protein diets and those of the 12% protein diet without amino acid addition. Data for mortality are given in Table 13.

FINAL BODY WEIGHT:

Body weights are expressed in kilograms, and they are a management tool to determine the adequacy of the nutrition provided. They determine whether the birds lost weight or gained weight, and also whether the diet provided adequate nutrition to support the egg production demands. There was no significant difference in body weights among hens on the different major sources of protein. There was also no effect of protein level. Data for final body weight are in Table 14.

TABLE 13. MORTALITY AS AFFECTED BY PROTEIN SOURCE AND LEVEL

(EXPERIMENT 1)

PROTEIN SOURCE	PROTEIN LEVEL				PROTEIN SOURCE MEAN
	16	14	12 WITHOUT AA	12 + AA (CONTROL)	
SOYBEAN MEAL	4.0 ^{b,a}	2.9 ^b	5.2 ^b	1.3 ^a	3.4 ^D
SUNFLOWER MEAL	5.0 ^{a,b}	1.0 ^a	3.1 ^a	3.8 ^b	3.2 ^D
MEAT AND BONE MEAL	3.6 ^a	4.6 ^c	4.9 ^b	4.3 ^b	4.4 ^D
PROTEIN LEVEL MEAN	4.2 ^B	2.8 ^A	4.4 ^B	3.1 ^{B,A}	

^{a,b,c} WITHIN COLUMNS, MEANS WITH UNLIKE SUPERSCRIPTS ARE SIGNIFICANTLY DIFFERENT (P<0.05)

^{A,B} MEANS WITH UNLIKE SUPERSCRIPTS ARE SIGNIFICANTLY DIFFERENT (P<0.05)

^D MEANS WITH SAME SUPERSCRIPTS ARE NONSIGNIFICANTLY DIFFERENT (P<0.05)

TABLE 14. FINAL BODY WEIGHT AS AFFECTED BY PROTEIN SOURCE AND LEVEL
(EXPERIMENT 1)

PROTEIN SOURCE	PROTEIN LEVEL, %				PROTEIN SOURCE MEAN
	16	14	12 WITHOUT AA	12 + AA (CONTROL)	
	Kg.	Kg.	Kg.	Kg.	Kg.
SOYBEAN MEAL	1.73 ^a	1.65 ^a	1.69 ^a	1.75 ^a	1.71 ^D
SUNFLOWER MEAL	1.70 ^a	1.70 ^a	1.68 ^a	1.70 ^a	1.71 ^D
MEAT AND BONE MEAL	1.72 ^a	1.68 ^a	1.73 ^a	1.71 ^a	1.70 ^D
PROTEIN LEVEL MEAN	1.72 ^A	1.68 ^A	1.70 ^A	1.72 ^A	---

WITHIN COLUMNS, MEANS WITH SAME SUPERSSCRIPTS ARE NONSIGNIFICANTLY DIFFERENT (P<0.05)

MEANS WITH SAME SUPERSSCRIPTS ARE NONSIGNIFICANTLY DIFFERENT (P<0.05)

D MEANS WITH SAME SUPERSSCRIPTS ARE NONSIGNIFICANTLY DIFFERENT (P<0.05)

BLOOD SERUM AMINO ACID CONCENTRATION

Both dietary protein source and level showed significant influences on serum amino acid concentrations ($P < 0.05$). However, there was no significant interaction between protein source and level on amino acid concentration.

Hens on the soybean meal diets showed significant superiority as to serum amino acid concentration to hens on either the meat and bone or sunflower meal diets for all amino acids analyzed except cystine and glycine ($P < 0.05$). Hens on meat and bone meal showed significantly higher serum amino acid concentrations in respect to lysine, glycine, alanine, proline, and aspartic acid as compared to hens on sunflower meal ($P < 0.05$), while hens on sunflower meal showed higher concentrations of the amino acids arginine, cystine, Isoleucine, valine, and threonine than hens on the meat and bone meal diets ($P < 0.05$). Hens on sunflower meal had significantly higher concentrations of serum cystine than hens on soybean meal ($P < 0.05$).

Hens on the 16% and the 14% protein levels showed significantly higher amino acid concentrations than those on the 12% protein diets with and without amino acid supplementation in respect to the amino acids, threonine, leucine, isoleucine, and valine ($P < 0.5$). There was no significant difference in serum concentrations in respect to histidine, glycine, and aspartic acid with all protein levels. The hens on 16% protein were superior as to serum proline concentration but were also inferior as to glutamic acid and serine concentration. The hens on the 12% protein diets supplemented with amino acids were

significantly superior as to lysine and alanine concentrations ($P < 0.05$); while the hens on the 12% protein diet without amino acid supplementation were significantly superior only as to serine ($P < 0.5$). Data for serum amino acid concentration as affected by protein level and source are shown respectively in Tables 15 and 16.

TABLE 15. BLOOD SERUM AMINO ACID CONTENT AS AFFECTED BY PROTEIN SOURCE

(EXPERIMENT 1)

AMINO ACIDS	PROTEIN SOURCE		
	SOYBEAN MEAL	MEAT AND BONE MEAL	SUNFLOWER MEAL
	µM/ml.	µM/ml.	µM/ml.
HISTIDINE	0.18 ^a	0.14 ^b	0.13 ^b
LYSINE	0.40 ^a	0.32 ^b	0.20 ^c
ARGININE	0.44 ^a	0.38 ^b	0.44 ^a
THREONINE	0.75 ^a	0.53 ^c	0.58 ^b
METHIONINE	0.09 ^a	0.08 ^a	0.09 ^a
HAUF-CYSTINE	0.43 ^b	0.45 ^b	0.60 ^a
PHENYLALANINE	0.14 ^a	0.12 ^a	0.13 ^a
TYROSINE	0.18 ^a	0.18 ^a	0.19 ^a
LEUCINE	0.34 ^a	0.25 ^b	0.27 ^b
ISOLEUCINE	0.13 ^a	0.08 ^c	0.11 ^b
VALINE	0.29 ^a	0.20 ^c	0.25 ^b
SERINE	0.71 ^a	0.56 ^a	0.64 ^a
GLYCINE	0.59 ^b	0.71 ^a	0.45 ^c
ALANINE	0.63 ^a	0.51 ^b	0.42 ^c
PROLINE	0.61 ^a	0.55 ^b	0.39 ^c
GLUTAMIC ACID	0.21 ^a	0.19 ^b	0.19 ^b
ASPARTIC ACID	0.78 ^a	0.65 ^a	0.48 ^b

a,b,c ACROSS THE ROWS, MEANS WITH UNLIKE SUPERSSCRIPTS ARE SIGNIFICANTLY DIFFERENT (P<0.05)

TABLE 16. BLOOD SERUM AMINO ACID CONTENT AS AFFECTED BY PROTEIN LEVEL

(EXPERIMENT 1)

AMINO ACIDS	PROTEIN LEVEL, %			
	16	14	12 WITHOUT AA	12 + AA
	$\mu\text{M/ml.}$	$\mu\text{M/ml.}$	$\mu\text{M/ml.}$	$\mu\text{M/ml.}$
HISTIDINE	0.15 ^a	0.15 ^a	0.16 ^a	0.16 ^a
LYSINE	0.32 ^b	0.31 ^b	0.21 ^c	0.40 ^a
ARGININE	0.48 ^a	0.45 ^{b,a}	0.39 ^{c,d}	0.37 ^c
THREONINE	0.71 ^a	0.71 ^a	0.61 ^b	0.46 ^c
METHIONINE	0.10 ^a	0.10 ^a	0.08 ^a	0.08 ^a
HALF-CYSTINE	0.45 ^{b,a}	0.54 ^a	0.47 ^b	0.48 ^{b,a}
PHENYLALANINE	0.13 ^a	0.13 ^a	0.13 ^a	0.12 ^a
TYROSINE	0.17 ^b	0.17 ^b	0.19 ^a	0.19 ^a
LEUCINE	0.30 ^a	0.30 ^a	0.27 ^b	0.27 ^b
ISOLEUCINE	0.13 ^a	0.12 ^a	0.10 ^b	0.09 ^b
VALINE	0.30 ^a	0.27 ^a	0.22 ^b	0.20 ^b
SERINE	0.56 ^c	0.61 ^{b,a}	0.77 ^a	0.59 ^{c,b}
GLYCINE	0.60 ^a	0.55 ^a	0.59 ^a	0.59 ^a
ALANINE	0.51 ^b	0.48 ^b	0.51 ^b	0.58 ^a
PROLINE	0.56 ^a	0.49 ^b	0.51 ^b	0.50 ^b
GLUTAMIC ACID	0.18 ^b	0.20 ^a	0.20 ^a	0.20 ^a
ASPARTIC ACID	0.64 ^a	0.67 ^a	0.60 ^a	0.64 ^a

^{a,b,c} ACROSS THE ROWS, MEANS WITH UNLIKE SUPERSCRIPTS ARE SIGNIFICANTLY DIFFERENT ($P < 0.05$)

EGG ALBUMEN AMINO ACIDS CONCENTRATION

There was a significant dietary protein influence on concentration of amino acids in egg albumen. Eggs from hens on the meat and bone meal diets showed significant superiority in egg albumen amino acid concentrations, which also reflected their superiority in Haugh units as compared to the soybean protein groups ($P < 0.05$). However, there was no significant difference between the egg albumen of hens fed meat and bone meal and those fed sunflower meal, and the egg albumen of hens fed sunflower or soybean meal diets were not significantly different. With the exception of cystine, eggs from hens fed meat and bone meal diets showed superiority to eggs from hens fed soy bean meal in amino acid concentration. Amino acid concentration of eggs from hens fed sunflower meal were intermediate between that of hens fed meat and bone meal and soybean meal, respectively. Egg albumen figures are shown in Table 17.

TABLE 17. EGG ALBUMIN AMINO ACID CONTENT AS AFFECTED BY PROTEIN SOURCE
(EXPERIMENT 1)

AMINO ACIDS	PROTEIN SOURCE		
	SOYBEAN MEAL	MEAT AND BONE MEAL	SUNFLOWER MEAL
	µM/gm.	µM/gm.	µM/gm.
HISTIDINE	14.0 ^b	18.0 ^a	16.4 ^{b,a}
LYSINE	49.9 ^b	56.5 ^a	51.1 ^{b,a}
ARGININE	33.7 ^b	39.7 ^a	36.3 ^{b,a}
THREONINE	35.8 ^b	43.3 ^a	39.3 ^{b,a}
METHIONINE	25.9 ^b	31.8 ^a	28.9 ^{b,a}
HALF-CYSTINE	65.2 ^a	54.6 ^a	53.9 ^a
PHENYLALANINE	37.1 ^b	44.7 ^a	40.8 ^{b,a}
TYROSINE	26.4 ^b	32.2 ^a	29.6 ^{b,a}
LEUCINE	65.9 ^b	78.2 ^a	71.3 ^{b,a}
ISOLEUCINE	4.0 ^b	4.7 ^a	4.2 ^{b,a}
VALINE	56.4 ^b	67.2 ^a	61.2 ^{b,a}
SERINE	63.3 ^b	73.6 ^a	66.7 ^{b,a}
GLYCINE	45.0 ^b	55.0 ^a	50.3 ^{b,a}
ALANINE	66.2 ^b	80.1 ^a	72.7 ^{b,a}
PROLINE	31.9 ^b	40.8 ^a	36.9 ^a
GLUTAMIC ACID	84.3 ^b	99.0 ^a	95.4 ^a
ASPARTIC ACID	74.8 ^b	91.5 ^a	81.8 ^b

a,b ACROSS THE ROWS, MEANS WITH UNLIKE SUPERSSCRIPTS ARE SIGNIFICANTLY DIFFERENT (P<0.05)

EXPERIMENT 2.

HEN-DAY EGG PRODUCTION

There was no significant difference in hen-day egg production between hens fed soybean or sunflower meal based diets in Experiment 2. The performance of hens for both dietary protein sources were very similar. Hens fed the meat and bone meal based diets still exhibited the poor egg production performance shown in Experiment 1. In this case it was significantly poorer ($P < 0.01$). Hens fed mixed diets of meat and bone meal with either soybean or sunflower meal or the sole supplement of sunflower or soybean meal was equally well.

On the basis of main effects due to protein level, the hens fed the 16% protein diets were significantly superior to those fed the 12% protein diets ($P < 0.01$). On the basis of main effects due to protein sources, hens on soybean meal, sunflower meal, and the mixed protein of sunflower plus meat and bone meal supported equal egg production performance though the performance of hens fed the sunflower meal diets was slightly better than that of hens fed soybean meal. The laying performance of hens on soybean meal and soybean plus meat and bone meal were essentially equal.

The egg production performance of hens fed mixed protein supplements of sunflower meal plus meat and bone meal was significantly higher than that of hens on soybean meal plus meat and bone meal ($P < 0.01$). Data on hen-day egg production are shown in Table 18.

TABLE 18. HEN-DAY EGG PRODUCTION AS AFFECTED BY PROTEIN SOURCE AND LEVEL
(EXPERIMENT 2)

PROTEIN SOURCE	PROTEIN LEVEL		
	16	12 (CONTROL)	PROTEIN SOURCE MEAN
SOYBEAN MEAL (SBM)	77.4 ^b	71.1 ^{b,c}	74.25 ^F
SUNFLOWER MEAL (SPM)	75.5 ^b	74.8 ^c	75.15 ^F
MEAT AND BONE MEAL (MBM)	65.8 ^a	43.3 ^a	54.55 ^D
SBM + MBM	73.4 ^b	66.3 ^b	69.85 ^E
SPM + MBM	76.8 ^b	71.5 ^{b,c}	74.15 ^F
PROTEIN LEVEL MEAN	73.78 ^B	65.4 ^A	-----

^{a,b,c} WITHIN COLUMNS, MEANS WITH UNLIKE SUPERSSCRIPTS ARE SIGNIFICANTLY DIFFERENT (P<0.01)

^{A,B} MEANS WITH UNLIKE SUPERSSCRIPTS ARE SIGNIFICANTLY DIFFERENT (P<0.01)

^{D,E,F} MEANS WITH UNLIKE SUPERSSCRIPTS ARE SIGNIFICANTLY DIFFERENT (P<0.01)

DAILY FEED CONSUMPTION AND FEED EFFICIENCY

Hens on sunflower meal consumed significantly more feed both at the 16% and 12% levels than hens on the other treatments ($P < 0.01$). Feed consumption was not significantly different among hens on soybean meal, soybean plus meat and bone meals, and sunflower plus meat and bone meal at the 16% protein level. Also, there was no significant difference between hens fed soybean meal and those fed sunflower plus meat and bone meal at the 12% protein level.

Feed consumption was significantly depressed for hens fed the meat and bone meal at both the 16 and 12% protein levels and for those fed the soybean plus meat and bone meal diet at the 12% level ($P < 0.01$). Main effects due to protein level showed that feed consumption was significantly depressed at the 12% protein level as compared to the 16% level ($P < 0.01$). Feed efficiency was significantly superior for hens fed soybean meal diets at both the 16 and 12% protein levels ($P < 0.01$). The hens on sunflower meal plus meat and bone meal showed feed efficiency equal to that of those on the soybean meal diets, and significantly better than the other diets ($P < 0.01$). Hens fed the sunflower meal diets had significantly poorer feed efficiencies compared to those fed the soybean meal diets ($P < 0.01$). There was no dietary influence of protein level seen in feed efficiency for hens fed the sunflower meal diets; nor was there any difference between the 16 and 12% protein levels respectively. There was no significant difference in feed efficiency among hens fed either sunflower meal, meat and bone meal, and soybean meal plus meat and bone meal diets

respectively, at 16% protein. Feed efficiency was significantly poorer for hens fed meat and bone meal at both the 16 and 12% protein levels ($P<0.01$) and also there was a significant influence of protein level for the meat and bone meal fed hens ($P<0.01$).

Feed efficiencies of hens as shown by main effects of protein source were similar among hens fed the soybean meal, soybean meal plus meat and bone meal, and sunflower meal plus meat and bone meal diets. As in Experiment 1, there was significant interaction between meat and bone meal protein level and feed consumption and feed efficiency, with a decline in feed consumption and feed efficiency being shown as protein level decreased ($P<0.01$). Data on feed consumption and feed efficiency are shown in Tables 19 and 20 respectively.

TABLE 19. DAILY FEED CONSUMPTION AS AFFECTED BY PROTEIN SOURCE AND LEVEL
(EXPERIMENT 2)

PROTEIN SOURCE	PROTEIN LEVEL		
	16	12 (CONTROL)	PROTEIN SOURCE MEAN
	gm.	gm.	gm.
SOYBEAN MEAL (SBM)	124.0 ^b	118.0 ^c	121.0 ^{E,F}
SUNFLOWER MEAL (SPM)	132.0 ^c	130.0 ^d	131.0 ^G
MEAT AND BONE MEAL (MBM)	114.0 ^a	99.0 ^a	106.0 ^D
SBM + MBM	122.0 ^b	111.0 ^b	117.0 ^E
SPM + MBM	122.0 ^b	121.0 ^c	122.0 ^F
PROTEIN LEVEL MEAN	123.0 ^B	116.0 ^A	

a,b,c,d WITHIN COLUMNS, MEANS WITH UNLIKE SUPERSCRIPTS ARE SIGNIFICANTLY DIFFERENT (P<0.01)

A,B MEANS WITH UNLIKE SUPERSCRIPTS ARE SIGNIFICANTLY DIFFERENT (P<0.01)

D,E,F,G MEANS WITH UNLIKE SUPERSCRIPTS ARE SIGNIFICANTLY DIFFERENT (P<0.01)

TABLE 20. FEED EFFICIENCY AS AFFECTED BY PROTEIN SOURCE AND LEVEL

(EXPERIMENT 2)

PROTEIN SOURCE	PROTEIN LEVEL		
	16	12 (CONTROL)	PROTEIN SOURCE MEAN
		(GMS EGGS/100 GMS FEED)	
SOYBEAN MEAL (SBM)	41 ^C	40 ^C	40 ^F
SUNFLOWER MEAL (SPM)	38 ^{a,b}	37 ^b	38 ^E
MEAT AND BONE MEAL (MBM)	37 ^a	27 ^a	32 ^D
SBM + MBM	39 ^b	38 ^b	39 ^{E,F}
SPM + MBM	41 ^C	38 ^b	39 ^{E,F}
PROTEIN LEVEL MEAN	39 ^A	36 ^B	--

a,b,c WITHIN COLUMNS, MEANS WITH UNLIKE SUPERSCRIPTS ARE SIGNIFICANTLY DIFFERENT (P<0.01)

A,B MEANS WITH UNLIKE SUPERSCRIPTS ARE SIGNIFICANTLY DIFFERENT (P<0.01)

D,E,F MEANS WITH UNLIKE SUPERSCRIPTS ARE SIGNIFICANTLY DIFFERENT (P<0.01)

EGG WEIGHT

Hens fed meat and bone meal diets laid significantly smaller size eggs than those fed either soybean meal or sunflower meal diets ($P < 0.05$). Hens fed either the sunflower meal diets or the soybean meal diets laid eggs that were similar in weight. Further, there was also no difference in egg weight between hens fed mixed diets of meat and bone meal with either sunflower or soybean meal; and neither was there any difference between eggs from hens fed the mixed diets or from hens fed the straight diets of either sunflower meal or soybean meal. There was no protein source and level interaction as to egg weight. Data on egg weights are shown in Table 21.

TABLE 21. EGG WEIGHT AS AFFECTED BY PROTEIN SOURCE AND LEVEL
(EXPERIMENT 2)

PROTEIN SOURCE	PROTEIN LEVEL		
	16	12 (CONTROL)	PROTEIN SOURCE MEAN
	gm.	gm.	gm.
SOYBEAN MEAL (SBM)	66.1 ^a	66.3 ^a	66.2 ^D
SUNFLOWER MEAL (SPM)	66.2 ^a	64.4 ^a	65.3 ^D
MEAT AND BONE MEAL (MBM)	62.9 ^b	62.0 ^b	62.5 ^E
SBM + MBM	64.6 ^{b,a}	63.6 ^{b,a}	64.6 ^D
SPM + MBM	65.0 ^a	64.8 ^a	64.9 ^D
PROTEIN LEVEL MEAN	65.0 ^A	64.0 ^A	---

a,b WITHIN COLUMNS, MEANS WITH UNLIKE SUPERSSCRIPTS ARE SIGNIFICANTLY DIFFERENT (P<0.05)

A MEANS WITH UNLIKE SUPERSSCRIPTS ARE SIGNIFICANTLY DIFFERENT (P<0.05)

D,E MEANS WITH UNLIKE SUPERSSCRIPTS ARE SIGNIFICANTLY DIFFERENT (P<0.05)

HAUGH UNITS AND EGG SHELL THICKNESS

Eggs from hens fed meat and bone meal diets showed superior Haugh units to eggs from hens fed the soybean meal diets at both the 16 and 12% protein levels. The values were similar for eggs from hens fed sunflower meal at the 16% protein level, but eggs from hens fed sunflower meal at the 12% protein level were significantly lower ($P < 0.01$). There was no difference in Haugh units among eggs from hens fed soybean, sunflower, and mixed diets of meat and bone meal with either soybean or sunflower at the 16% protein level. There was no difference between the Haugh units of eggs from hens fed sunflower meal diets and hens fed mixed diet of sunflower plus meat and bone meal at the 12% protein level. There was significant difference between eggs from hens fed soybean meal diets and hens fed mixed diet of soybean plus meat and bone meal in Haugh units at the 12% protein level ($P < 0.01$). Influence of main effect of treatment due to protein source was significant; the meat and bone meal protein source fed hens, eggs from the meat and bone protein source fed hens were superior in Haugh units compared to either those from sunflower or soybean protein sources respectively ($P > 0.01$). Also, main effect due to protein level as to Haugh units was significantly higher at the 12% level than at the 16% level. However, there was no influence of protein source x protein level interaction on Haugh units. Data for Haugh units are presented in Table 22.

There was no significant difference and even any worthwhile numerical difference among hens for egg shell thickness in all dietary

treatments. Thus neither protein level nor protein source had any dietary effect on egg shell thickness. Data for egg shell thickness are shown in Table 23.

Treatment	Egg Shell Thickness (mm)	
	Mean	Standard Error
Control	0.35	0.01
Casein 12%	0.35	0.01
Casein 18%	0.35	0.01
Casein 24%	0.35	0.01
Casein 30%	0.35	0.01
Casein 36%	0.35	0.01
Casein 42%	0.35	0.01
Casein 48%	0.35	0.01
Casein 54%	0.35	0.01
Casein 60%	0.35	0.01
Casein 66%	0.35	0.01
Casein 72%	0.35	0.01
Casein 78%	0.35	0.01
Casein 84%	0.35	0.01
Casein 90%	0.35	0.01
Casein 96%	0.35	0.01
Casein 100%	0.35	0.01

TABLE 22. HAUGH UNITS AS AFFECTED BY PROTEIN SOURCE AND LEVEL
(EXPERIMENT 2)

PROTEIN SOURCE	PROTEIN LEVEL		PROTEIN SOURCE MEAN
	16	12 (CONTROL)	
SOYBEAN MEAN (SEM)	79.2 ^a	79.5 ^a	79.3 ^D
SUNFLOWER MEAL (SPM)	80.5 ^{a,b}	81.1 ^b	80.8 ^E
MEAT AND BONE MEAL (MEM)	81.6 ^b	84.5 ^d	83.1 ^F
SEM + MEM	79.2 ^a	82.5 ^c	80.9 ^E
SPM + MEM	79.6 ^a	81.2 ^b	80.4 ^{E,D}
PROTEIN LEVEL MEAN	79.3 ^B	80.3 ^A	---

a,b,c,d WITHIN COLUMNS, MEANS WITH UNLIKE SUPERSSCRIPTS ARE SIGNIFICANTLY DIFFERENT (P<0.01)

A,B MEANS WITH UNLIKE SUPERSSCRIPTS ARE SIGNIFICANTLY DIFFERENT (P<0.01)

E,D,F MEANS WITH UNLIKE SUPERSSCRIPTS ARE SIGNIFICANTLY DIFFERENT (P<0.01)

TABLE 23. EGG SHELL THICKNESS AS AFFECTED BY PROTEIN SOURCE AND LEVEL.
(EXPERIMENT 2)

PROTEIN SOURCE	PROTEIN LEVEL		
	16	12 (CONTROL)	PROTEIN SOURCE MEAN
	mm x 100	mm x 100	mm x 100
SOYBEAN MEAL (SBM)	38.3 ^a	38.0 ^a	38.2 ^D
SUNFLOWER MEAL (SPM)	38.3 ^a	38.0 ^a	38.2 ^D
MEAT AND BONE MEAL (MBM)	37.3 ^a	38.5 ^a	38.2 ^D
SBM + MBM	37.4 ^a	38.0 ^a	37.7 ^D
SPM + MBM	37.7 ^a	38.0 ^a	37.9 ^D
PROTEIN LEVEL MEAN	37.8 ^A	38.1 ^A	---

^a WITHIN COLUMNS, MEANS WITH SAME SUPERSSCRIPTS ARE NONSIGNIFICANTLY DIFFERENT (P<0.05)

^A MEANS WITH SAME SUPERSSCRIPTS ARE NONSIGNIFICANTLY DIFFERENT (P<0.05)

^D MEANS WITH SAME SUPERSSCRIPTS ARE NONSIGNIFICANTLY DIFFERENT (P<0.05)

MORTALITY

Hens on the soybean meal plus meat and bone meal diet at the 16% protein level showed less mortality compared to hens on the other 16% protein diets ($P < 0.01$). There were no significant differences in mortality rate among hens on soybean, meat and bone, and the mixed diet of sunflower plus meat and bone meal at the 16% protein level. Mortality was highest for sunflower meal fed hens at the 16% protein level, and it was significantly different from the other diets at the same level ($P < 0.01$).

However, at the 12% protein level, hens on mixtures of sunflower meal plus meat and bone meal showed a mortality rate comparable to the other diets at the same level; and this was significant at ($P < 0.01$). There were no significant differences in mortality rate among hens on soybean, sunflower, meat and bone, and mixtures of soybean meal and meat and bone meal at the 12% protein level.

On the main effect due to protein level basis, hens fed the 12% protein level diets showed the least mortality compared to the hens on the 16% protein diets ($P < 0.01$). On the main effect due to protein source basis, hens on the mixed diets of either meat and bone meal with soybean or sunflower showed the least mortality rate as compared to those on other diets ($P < 0.01$). There were no significant differences in mortality among hens on soybean, sunflower, or meat and bone meal diets. However, mortality was numerically higher in hens fed sunflower meal diets as compared to those fed either soybean or meat and bone

meal diets; and it was also numerically higher in hens fed meat and bone meal diets as compared to those fed soybean meal diets.

There was no attributable cause of mortality due to dietary effect, as far as post-mortem results from the Veterinary Department indicated. There was also no evidence of an interaction between protein source and level on mortality. Mortality figures are given in Table 24.

TABLE 24. MORTALITY AS AFFECTED BY PROTEIN SOURCE AND LEVEL

(EXPERIMENT 2)

PROTEIN SOURCE	PROTEIN LEVEL		
	16	12 (CONTROL)	PROTEIN SOURCE MEAN
SOYBEAN MEAL (SBM)	10.4 ^b	6.4 ^b	8.4 ^{E,D}
SUNFLOWER MEAL (SPM)	13.5 ^c	6.6 ^b	10.1 ^E
MEAT AND BONE MEAL (MBM)	10.4 ^b	7.8 ^b	9.1 ^E
SBM + MBM	7.3 ^a	7.3 ^b	7.3 ^D
SPM + MBM	9.9 ^b	4.6 ^a	7.30 ^D
PROTEIN LEVEL MEAN	10.3 ^B	6.5 ^A	-----

^{a,b,c} WITHIN COLUMNS, MEANS WITH UNLIKE SUPERSCRIPTS ARE SIGNIFICANTLY DIFFERENT (P<0.01)

^{A,B} MEANS WITH UNLIKE SUPERSCRIPTS ARE SIGNIFICANTLY DIFFERENT (P<0.01)

^{D,E,F} MEANS WITH UNLIKE SUPERSCRIPTS ARE SIGNIFICANTLY DIFFERENT (P<0.01)

BODY WEIGHT AT PEAK EGG PRODUCTION AND FINAL BODY WEIGHT

The meat and bone diets at both 16 and 12% protein levels and the mixed diets of soybean meal plus meat and bone meal failed to maintain the hens' body weight at peak egg production, and it was significant at ($P < 0.05$). There was no significant difference among hens on the other dietary treatments at the 16% protein level.

On the main effect due to protein level basis, the 12% protein level fed hens were significantly lighter than those fed the 16% protein level at peak egg production ($P < 0.01$). On the main effect due to protein source basis, hens on meat and bone meal were significantly lighter than hens on the other diets except for those on mixtures of soybean meal plus meat and bone meal ($P < 0.01$). However, there was no significant difference among hens in all dietary treatments with regards to final body weight. Data for body weight at peak egg production and final body weight are shown in Tables 25 and 26 respectively.

TABLE 25. BODY WEIGHT AT PEAK PRODUCTION, AS AFFECTED BY PROTEIN SOURCE AND LEVEL
(EXPERIMENT 2)

PROTEIN SOURCE	PROTEIN LEVEL		
	16	12 (CONTROL)	PROTEIN SOURCE MEAN
	Kg.	Kg.	Kg.
SOYBEAN MEAL (SBM)	1.76 ^b	1.76 ^c	1.76 ^F
SUNFLOWER MEAL (SPM)	1.75 ^b	1.70 ^c	1.73 ^F
MEAT AND BONE MEAL (MBM)	1.60 ^a	1.48 ^a	1.54 ^D
SBM + MBM	1.78 ^b	1.55 ^{b,a}	1.67 ^E
SPM + MBM	1.80 ^b	1.66 ^b	1.73 ^F
PROTEIN LEVEL MEAN	1.74 ^B	1.63 ^A	---

a,b,c WITHIN COLUMNS, MEANS WITH UNLIKE SUPERSSCRIPTS ARE SIGNIFICANTLY DIFFERENT (P<0.05)

A,B MEANS WITH UNLIKE SUPERSSCRIPTS ARE SIGNIFICANTLY DIFFERENT (P<0.01)

D,E,F MEANS WITH UNLIKE SUPERSSCRIPTS ARE SIGNIFICANTLY DIFFERENT (P<0.01)

TABLE 26. FINAL BODY WEIGHT AS AFFECTED BY PROTEIN SOURCE
(EXPERIMENT 2)

PROTEIN SOURCE	AVERAGE FINAL BODY WEIGHT (KG)
SOYBEAN MEAL (SBM)	1.80 ^a
SUNFLOWER MEAL (SPM)	1.73 ^a
MEAT AND BONE MEAL (MBM)	1.71 ^a
SBM + MBM	1.73 ^a
SPM + MBM	1.76 ^a

^a MEANS WITH THE SAME SUPERSSCRIPTS ARE NONSIGNIFICANTLY DIFFERENT
(P<0.05)

BLOOD SERUM AMINO ACID CONCENTRATION

Except for the amino acids threonine, valine, and glycine, there was no significant difference in serum amino acid concentration among hens due to dietary protein levels. The above amino acids were more significantly concentrated in hens fed at the 16% dietary protein level than in those at the 12% protein level ($P < 0.05$). Sunflower meal fed hens had significantly higher serum amino acid concentrations of histidine, arginine, threonine, and valine than those on the other diets, while hens fed soybean meal diets had significantly higher concentrations of lysine and aspartic acid compared to the others ($P < 0.05$). Meat and bone meal fed hens were only significantly higher in serine concentration, while hens on the mixtures of sunflower and meat and bone meal were high in glycine concentration. Data for serum amino acid concentration as influenced by protein source and level are shown in Tables 27 and 28 respectively.

TABLE 27. BLOOD SERUM AMINO ACID CONTENT AS AFFECTED BY PROTEIN SOURCE
(EXPERIMENT 2)

AMINO ACIDS	PROTEIN SOURCE				
	SOYBEAN MEAL (SBM)	SUNFLOWER MEAL (SPM)	MEAT AND BONE MEAL	SBM + MBM	SPM + MBM
	µM/ml.	µM/ml.	µM/ml.	µM/ml.	µM/ml.
HISTIDINE	0.14 ^b	0.15 ^a	0.13 ^{c,b}	0.12 ^c	0.13 ^{c,b}
LYSINE	0.32 ^a	0.26 ^b	0.26 ^b	0.28 ^b	0.23 ^c
ARGININE	0.33 ^c	0.47 ^a	0.35 ^c	0.38 ^{c,b}	0.41 ^b
THREONINE	0.51 ^b	0.64 ^a	0.45 ^c	0.50 ^b	0.52 ^b
METHIONINE	0.10 ^a	0.10 ^a	0.08 ^b	0.08 ^b	0.09 ^{b,a}
HALF-CYSTINE	0.39 ^b	0.55 ^a	0.33 ^c	0.35 ^c	0.44 ^b
PHENYLALANINE	0.12 ^a	0.12 ^a	0.10 ^a	0.10 ^a	0.10 ^a
TYROSINE	0.14 ^{b,a}	0.13 ^{b,c}	0.13 ^{b,c}	0.15 ^a	0.12 ^c
LEUCINE	0.33 ^a	0.27 ^{b,a}	0.24 ^b	0.28 ^{b,a}	0.29 ^{b,a}
ISOLEUCINE	0.12 ^a	0.13 ^a	0.08 ^c	0.10 ^b	0.10 ^b
VALINE	0.26 ^b	0.30 ^a	0.20 ^c	0.25 ^b	0.26 ^b
SERINE	0.64 ^b	0.53 ^b	0.72 ^a	0.66 ^b	0.64 ^b
GLYCINE	0.50 ^c	0.47 ^d	0.66 ^b	0.63 ^b	0.72 ^a
ALANINE	0.46 ^a	0.42 ^{b,a}	0.44 ^{b,a}	0.41 ^b	0.43 ^{b,a}
PROLINE	0.54 ^{c,d}	0.49 ^d	0.57 ^{c,b}	0.60 ^b	0.68 ^a
GLUTAMIC ACID	0.23 ^a	0.21 ^a	0.21 ^a	0.20 ^a	0.21 ^a
ASPARTIC ACID	1.33 ^a	1.08 ^b	1.09 ^b	0.40 ^b	1.15 ^b
TRYPTOPHAN	0.06 ^a	0.06 ^a	0.04 ^b	0.51 ^a	0.09 ^a

a,b,c,d ACROSS THE ROWS, MEANS WITH UNLIKE SUPERSSCRIPTS ARE SIGNIFICANTLY DIFFERENT (P<0.05)

TABLE 28. BLOOD SERUM AMINO ACID CONTENT AS AFFECTED BY PROTEIN LEVEL

(EXPERIMENT 2)

AMINO ACIDS	PROTEIN LEVEL	
	16	12
	$\mu\text{M/ml.}$	$\mu\text{M/ml.}$
HISTIDINE	0.13 ^a	0.14 ^a
LYSINE	0.25 ^a	0.29 ^a
ARGININE	0.39 ^a	0.39 ^a
THREONINE	0.59 ^a	0.47 ^b
METHIONINE	0.08 ^a	0.09 ^a
HALF-CYSTINE	0.04 ^a	0.04 ^a
PHENYLALANINE	0.11 ^a	0.11 ^a
TYROSINE	0.13 ^a	0.15 ^a
LEUCINE	0.3 ^a	0.10 ^a
ISOLEUCINE	0.11 ^a	0.10 ^a
VALINE	0.29 ^a	0.21 ^b
SERINE	0.04 ^a	0.65 ^a
GLYCINE	0.61 ^a	0.58 ^b
ALANINE	0.32 ^a	0.44 ^a
PROLINE	0.60 ^a	0.55 ^a
GLUTAMIC ACID	0.20 ^a	0.22 ^a
ASPARTIC ACID	0.20 ^a	0.22 ^a
TRYPTOPHAN	0.05 ^a	0.07 ^a

^{a, b} ACROSS THE ROWS, MEANS WITH UNLIKE SUPERSSCRIPTS ARE SIGNIFICANTLY DIFFERENT ($P < 0.05$)

EGG ALBUMEN AMINO ACID CONCENTRATION

Hens fed the mixtures of soybean plus meat and bone meal showed significant superiority in concentration of all amino acids analyzed except cystine, leucine, valine, alanine and proline ($P < 0.05$) compared to hens on the other diets. Egg albumen of hens fed meat and bone meal diets were not significantly different from hens fed the mixed diet of soybean plus meat and bone meal in amino acid concentration, except for histidine, arginine, methionine, glycine, and aspartic acid. Further, there was no significant difference among hens fed soybean, sunflower, and the mixed diet of sunflower plus meat and bone meal in amino acid concentration, except that, hens on the sunflower meal diets showed a significantly lower glutamic acid content ($P < 0.05$). Figures for egg albumen amino acid concentration are shown in Table 29.

TABLE 29. EGG ALBUMEN AMINO ACID CONTENT AS AFFECTED BY PROTEIN SOURCE

(EXPERIMENT 2)

AMINO ACIDS	PROTEIN SOURCE				
	SOYBEAN MEAL	SUNFLOWER MEAL	MEAT AND BONE MEAL	SBM + MBM	SFM + MBM
	$\mu\text{M/gm.}$	$\mu\text{M/gm.}$	$\mu\text{M/gm.}$	$\mu\text{M/gm.}$	$\mu\text{M/gm.}$
HISTIDINE	16.3 ^b	16.5 ^b	18.4 ^a	16.8 ^b	15.3 ^b
LYSINE	51.3 ^b	51.0 ^b	58.5 ^a	53.8 ^{b,a}	49.1 ^b
ARGININE	37.3 ^b	35.7 ^b	41.5 ^a	37.8 ^{b,a}	34.9 ^b
THREONINE	38.8 ^b	40.0 ^b	45.5 ^a	39.8 ^b	37.2 ^b
METHIONINE	28.3 ^b	26.7 ^b	32.1 ^a	29.3 ^b	27.5 ^b
HALF-CYSTINE	66.2 ^a	65.6 ^a	68.0 ^a	66.8 ^a	65.0 ^a
PHENYLALANINE	40.6 ^b	41.5 ^b	46.9 ^a	42.7 ^{b,a}	39.0 ^b
TYROSINE	30.0 ^b	29.9 ^b	33.4 ^a	31.2 ^{b,a}	29.0 ^b
LEUCINE	74.3 ^{b,a}	70.5 ^b	80.4 ^a	74.4 ^{b,a}	68.1 ^b
ISOLEUCINE	4.2 ^{b,c}	4.2 ^{b,c}	4.7 ^a	4.4 ^{b,a}	4.0 ^c
VALINE	62.2 ^{b,a}	62.0 ^{b,a}	68.8 ^a	64.1 ^{b,a}	58.1 ^b
SERINE	68.0 ^b	67.0 ^b	76.0 ^a	69.8 ^{b,a}	67.0 ^b
GLYCINE	50.0 ^b	50.3 ^b	56.9 ^a	51.5 ^b	47.5 ^b
ALANINE	75.7 ^{b,a}	73.4 ^{b,a}	82.1 ^a	75.7 ^{b,a}	70.5 ^b
PROLINE	37.4 ^{b,a}	40.0 ^{b,a}	45.7 ^a	36.5 ^{b,a}	34.5 ^b
GLUTAMIC ACID	94.5 ^b	95.5 ^{b,a}	99.9 ^b	96.9 ^{b,a}	88.7 ^c
ASPARTIC ACID	82.9 ^b	84.5 ^b	95.1 ^a	85.2 ^b	79.5 ^b

a,b,c ACROSS THE ROWS, MEANS WITH UNLIKE SUPERSSCRIPTS ARE SIGNIFICANTLY DIFFERENT (P<0.05)

DISCUSSION

Hen-day egg production data as indicated in Experiments 1 and 2 in respect to soybean meal and sunflower meal diets showed that the 12% protein supported egg production as satisfactorily as 16% protein, provided it is supplemented with lysine and methionine. These results agree with those of Lewis (1966), Kashani (1975), and Eldred *et al.* (1975). However, because of the near adequacy of protein requirement provided by the 12% protein level from both soybean meal and sunflower meal, the response to supplementary methionine and lysine was neither significant nor consistent. According to Bray (1960); Waibel and Johnson (1961); Adams *et al.* (1961); and Bray and Morrissey (1962); a 12% protein diet contains a marginal protein level for furnishing the amino acid requirements of laying hens. Therefore, the biggest problem of such a protein level is one of adequately supplementing it to provide all essential amino acids for egg production. There was significant difference between the 16% and the 14% protein in supporting hen-day egg production performance as shown in Experiment 1 with regards to the data for soybean meal and sunflower meal. This result disagreed with that of Ross and Herrick (1976) who showed no significant difference between hens on the 16% and 14% protein as to hen-day egg production performance. Under the conditions of this experiment, hens on the 14% protein for both the soybean meal and sunflower meal diets significantly outperformed those on 16% protein ($P < 0.05$).

There was a significant interaction between protein level and egg production performance with regards to meat and bone meal in the

data shown for the two experiments ($P < 0.01$). Egg production performance declined progressively with each decline in protein level. The possible explanation may be a protein inadequacy at the lower levels to furnish necessary amino acids for egg protein synthesis, coupled with low availability of amino acids unduly delayed in being released to be used. The poor egg production performance of hens fed meat and bone meal diets cannot be reasonably attributed to a deficiency of essential amino acids or high calcium and phosphorus. If so, the effect could have been seen equally in the 16% protein level, and above all a total cease or pause with egg production during the experimental periods should have been noticed. For according to Johnson and Fisher (1956), and Fisher et al. (1957), a deficiency in essential dietary amino acid levels should ultimately result in cessation of egg production or at least a pause.

However, the mixed diets of meat and bone meal with either soybean meal or sunflower meal supported hen-day egg production performance equal to either sunflower or soybean meal respectively. This result agrees with the recommendations of McDonald et al. (1973), that meat and bone meal makes an excellent protein source in combination with other protein supplements in supporting production performance either in swine or poultry.

Critical evaluation of the data in the two experiments reflecting egg production performance indicates that the nutritive value of sunflower meal is equal to soybean meal as long as "energy and lysine factors" are balanced for sunflower meal. Secondly, a sunflower meal

of at least 32% protein or more needs to be used to achieve a comparable performance level with soybean meal. The overall consideration of the data from the two experiments agrees with reports of Walter et al. (1951), who reported satisfactory egg production performance with sunflower meal in a comparison study with soybean meal; and Hale and Brown (1957) who reported a similar finding in sunflower diets for laying hens. Further, the results also agree with those of Uwayjan et al. (1983). Though Uwayjan et al. (1983) fed sunflower seeds, instead of sunflower meal, and reported no significant difference between sunflower meal and soybean meal.

However, my results disagree with those of Rose et al. (1972) who reported an adverse egg production performance ($P < 0.01$) of hens from 100% replacement of soybean meal with sunflower meal with a slightly reduced egg production performance at 50% replacement. There was no incidence of egg staining shown by the Rose et al. (1972) report.

The data from the two experiments show that hens on sunflower meal diets consume significantly more feed than hens on either soybean or meat and bone meal diets. Because sunflower meal is low in energy and high in fibre, it is naturally expected that hens would eat more feed to meet the energy requirement. This is reflected in the experimental data. This finding agrees with those of Hale and Brown (1957); Walter et al. (1959); and Rose et al. (1972). However, Uwayjan et al. (1983), reported a drop in feed intake with higher levels of sunflower seeds in diets for laying hens. This agrees with

similar findings of Dinusson et al. (1980) in swine nutrition where feed intake was depressed by a 39% sunflower seed addition in a growing-finishing swine diet. The data from both experiments indicate no dietary protein source x protein level interaction influence on hens fed soybean and sunflower meal diets. Meat and bone meal fed hens in both experiments did show depressed feed intake, with a progressive decline as protein level decreased. There was evidence of a significant protein level and feed intake interaction ($P < 0.01$).

The data from the two experiments show significant differences between hens fed soybean and sunflower meal diets in feed efficiency ($P < 0.01$). This result agrees with the report of Rose et al. (1972), but is in disagreement with Walter et al. (1959), and Uwayjan et al. (1983).

Egg weight data in both experiments showed that there was no significant difference between weight of eggs from hens fed sunflower meal diets and those fed soybean meal diets. Hens fed meat and bone meal diets laid significantly smaller size eggs than either of those fed sunflower meal or soybean meal diets. The data on egg weight from both experiments with regards to hens fed sunflower meal and soybean meal diets agree with those of Pettit et al. (1944); Hale and Brown (1957); Walter et al. (1959); Rose et al. (1972); and Uwayjan et al. (1983) respectively.

Further observation of the experimental data suggests that there was no influence of dietary protein level on egg size except for egg size of hens fed the meat and bone meal diets. This observation

appears to disagree with earlier reports of Johnson and Fisher (1959), who reported significantly heavier eggs from hens fed a 15.7% protein level versus either 10.4 or 11.2% protein levels, respectively. However, it must be pointed out here that Johnson and Fisher's comparison was made with lower protein levels (10.4 and 11.3%). Such low protein levels are inadequate to furnish enough protein per se and necessary amino acids for adequate egg protein synthesis. One of the greatest factors that affect egg size is protein adequacy, since the dry matter of an egg is 50% protein.

However, the findings here are in direct conflict with those of Quisenberry and Bradley (1962), who reported an influence of dietary protein level on egg size. They studied the effect of dietary protein levels of 17%, 15%, and 13% on egg size, feed efficiency, final body weight, and mortality. They indicated that higher protein levels gave heavier eggs with better feed efficiency, but with no effect on mortality and final body weight. My findings also disagree with those of Thornton et al. (1956); who also reported a reduced egg size with decreases in protein levels.

Although hens fed meat and bone meal diets laid eggs of smaller size compared to those fed either soybean or sunflower meal diets; none-the-less, the egg size was within grade "A Large", according to the standards set by the United States Department of Agriculture.

The data from both experiments show that hens fed meat and bone meal diets laid eggs significantly higher in Haugh unit values than

those fed soybean meal diets but equal to those fed sunflower meal diets.

The data for Experiment 1 do not show a significant main effect of dietary protein level on Haugh unit values. The hens fed 12% protein diets produced eggs showing higher Haugh unit values than those fed the higher protein levels of 16 or 14%. The Haugh unit values of Experiment 2 clearly show the influence of dietary protein level. Eggs from hens fed 12% protein show significantly higher Haugh unit values than those from hens fed the 16% protein level. This finding is in agreement with the finding of Deaton and Quisenberry (1965) who reported higher Haugh unit scores for birds on lower than higher protein diets (17% versus 14%).

There was no significant difference in egg shell thickness among hens fed soybean, sunflower or meat and bone meal diets in Experiment 1 and the mixed diets of meat and bone meal with either sunflower or soybean meal in Experiment 2. However, in Experiment 2 the shell thickness of eggs from hens fed the sunflower meal diets and the mixed diets of sunflower with meat and bone meal were slightly reduced as compared to that of hens on the other dietary treatments.

Mortality data showed no significant dietary protein level or source influences in Experiment 1, but in Experiment 2 the hens on sunflower meal diets showed slightly more mortality than the other groups. However, the mortality data were within an acceptable normal average for the laying year. The hens on the mixed diets of either sunflower or soybean meal with meat and bone meal showed the least

mortality. The overall data on mortality from the two experiments agree with those reported by Pettit et al. (1944); Walter et al. (1957); Rose et al. (1972); and Uwayjan et al. (1983).

The final body weights of hens in both experiments show no significant difference among dietary treatments. However, the meat and bone meal diets failed to maintain body weight at peak egg production.

The results with regards to final body weights between hens fed soybean and sunflower diets disagree with those reported by Hale and Brown (1957), and Rose et al. (1972); however, they are in agreement with the findings of Walter et al. (1957), and Uwayjan et al. (1983).

Serum amino acid concentration data were analyzed statistically with respect to protein level and source influences. With regards to protein source in Experiment 1, hens fed soybean diets showed a higher concentration of all amino acids with the exception of cystine and glycine. This confirms the known fact that the sulfur amino acids are the first limiting amino acids in soybean protein and that either cystine or methionine supplementation rectifies such a deficiency (McGinnis et al. 1948). The low concentration of cystine was also seen in hens fed meat and bone meal protein, which again is naturally low in sulfur amino acids (Kratzer and Davis, 1959). Concentration of cystine was higher in sunflower meal fed hens ($P < 0.05$) as compared to soybean and meat and bone meal fed hens. Here again, this high concentration follows the pattern of sunflower protein amino acid composition (McGinnis et al. 1948; Alexander and Hill, 1952; and Harvey, 1970). Serum lysine concentration, as expected, was lowest in sunflower meal

fed hens followed by the values for meat and bone meal fed hens and the highest values for soybean meal fed hens. Serum concentrations of arginine, threonine, and isoleucine were low for meat and bone meal fed hens. This agrees with the report of McDonald et al (1973). Also the concentration of threonine was low for sunflower meal fed hens, which seems to agree with the suggestion of Cuca et al. (1973) that, threonine could be a second possible limiting amino acid in sunflower protein. The pattern of serum amino acid concentration in general, was the same in both experiments with regard to the protein source influence.

The concentrations of serum amino acids do not indicate a consistent pattern in these experiments with regard to the effect of dietary protein level as seen in Experiment 1. A higher concentration of lysine was noted with the 12% protein amino acid supplemented control group, followed by the 16 and 14% protein levels. The lowest lysine values were shown by the 12% protein level without amino acid addition. Arginine showed a decreasing concentration with a decline in protein level. The concentrations of histidine, phenylalanine, and aspartic acid did not show any influence of dietary protein level. All the other amino acids either were increased by higher protein levels or were increased by low protein levels and decreased by higher protein levels, respectively. However, consistent decreases in leucine, isoleucine, and valine were noticed as being influenced by dietary protein level. This, in general, agreed with the findings of Kashani (1978). This effect was seen in Experiment 1. However, in Experiment

2 the only serum amino acids concentrations which were influenced by dietary protein levels were threonine, valine, and glycine. In Experiment 2 only two replicate samples were taken and analyzed, and treatment levels were also only two; so statistically there were not enough levels and replications to indicate meaningful results with the data obtained.

Egg albumen amino acid concentrations reflect the influence of dietary protein source on the Haugh unit values. In both experiments hens fed meat and bone meal diets showed significantly superior egg albumen amino acid concentration as compared to those fed soybean meal diets. Hens fed sunflower meal diets also seemed to show numerically higher egg albumen amino acid concentrations than those fed soybean meal diets. There was no significant difference in egg albumen amino acid concentration among hens fed mixed diets of meat and bone meal with either sunflower or soybean meal. Egg albumen concentration of cystine was the only amino acid that did not vary due to dietary protein source. Aspartic acid egg albumen concentrations were also similar among hens for all dietary treatments, as were those for threonine and arginine.

CONCLUSIONS

The following conclusions are drawn from the data collected in the two experiments to evaluate the nutritional value of soybean, sunflower, and meat and bone meals for egg production as determined by the parameters measured:

1. Sunflower meal and soybean meal have equal nutritional value in practical diets for laying hens, provided the "lysine" and "energy" factors are corrected for sunflower meal.
2. Meat and bone meal as a major source is a poor protein supplement in practical diets for satisfactory egg laying performance.
3. Hens fed mixed diets of meat and bone meal with either soybean meal in a ratio of 4:1 or 1:1, or with sunflower meal in a ratio of 3:1 or 0.6:1 gave equally satisfactory egg production performance, comparable to the performance of hens fed either sunflower meal or soybean meal diets.
4. Feed consumption was significantly higher for hens fed sunflower meal diets, with relatively poorer feed efficiency as compared to that of hens on soybean meal diets.
5. Feed consumption was significantly depressed by hens fed meat and bone meal diets with significantly poorer feed efficiency compared to that of either hens fed sunflower meal diets or soybean meal diets.
6. Hens fed meat and bone meal diets laid smaller size eggs compared to hens fed either soybean meal diets or sunflower meal diets. However, the egg size laid by hens on meat and bone meal diets was

within grade "A Large", according to United States Department of Agriculture standards.

7. Albumen quality of eggs laid by hens fed meat and bone meal was significantly higher than that of eggs laid by hens fed soybean meal diets.

8. There were no differences in final body weight due either to influence of dietary protein source or level. However, hens fed meat and bone meal diets showed significantly reduced body weight during peak egg production as compared to hens fed either sunflower meal or soybean meal diets.

9. No differences were noted in mortality and egg shell thickness for all dietary treatments.

10. The overall poor performance of meat and bone meal as a major source of protein in practical laying diets cannot be possibly attributed to either a gross deficiency of essential amino acids or high calcium and phosphorus levels. The probable cause may be either an amino acid imbalance or a very low or slow availability of essential amino acids for egg protein synthesis.

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